ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1971
PART I

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

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NATIONAL CANCER INSTITUTE

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NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1970 through June 30, 1971

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ANNUAL REPORT
NATIONAL CANCER INSTITUTE
July 1, 1970 - June 30, 1971

Introduction

The National Cancer Institute was occupied very largely during the year with preparations for mounting a greatly expanded and intensified Federal effort to bring cancer under effective control in the decade of the seventies.

Indications that the trend toward retrenchment in cancer research funding during the fiscal year 1970 would be eased appeared with the President's 1971 appropriation request of $202,383,000 for the Institute. The appropriation act increased this by $28 million, and the whole amount was apportioned by the Office of Management and Budget, giving an operating level approximately $50 million above that of the previous year. The President then requested an appropriation of $232,234,000 for fiscal 1972, and in his State of the Union Message in January he called for an extra $100 million for cancer research, and said he would ask later for any additional funds that could be effectively used.

In this message President Nixon listed the conquest of cancer among the most important national goals put forward by his Administration, and urged a national commitment to this goal as had been made to the goal of placing a man on the moon in the decade of the sixties. In his Health Message delivered to the Congress in February he spoke further on the need for an expanded and coordinated Federal effort to conquer cancer, and on May 11 announced that he had sent to the Congress draft legislation to establish, within the National Institutes of Health, a Cancer Cure Program under a director appointed by and responsible to the President.

As planning went forward for an expanded Federal cancer research effort involving the National Cancer Institute, bills were also under consideration in Congress to establish an independent National Cancer Authority which would absorb NCI and mount an intensive effort to make major gains against cancer within the present decade. Interest centered on S. 34, introduced in the 92nd Congress by Senator Kennedy of Massachusetts. This bill incorporated the recommendations of a National Panel of Consultants on the Conquest of Cancer which had been appointed under a resolution introduced by Senator Yarborough, Chairman of the Senate Committee on Labor and Public Welfare in the 91st Congress. It proposed a cancer research operating level of approximately $400 million for fiscal 1972 and $800 million to $1 billion by 1976.

The National Cancer Institute gave full cooperation to the National Panel of Consultants on the Conquest of Cancer, which was chaired by Mr. Benno C. Schmidt, Managing Partner, J. E. Whitney & Co., New York City, and co-chaired by Dr. Sidney Farber, Director of Research, Children's Cancer Research Foundation, Boston. A staff appointed by the Panel to perform research for and assist in the preparation of its report was given office space in Building 31 and provided all assistance requested in the performance of its duties.

Meanwhile, the Institute Director and staff carried forward a series of program reviews and evaluations in preparation for the new, large Federal cancer effort foreshadowed by the developments within the Administration and the Congress. The Institute's senior management staff began a series of seminars on the systems approach to the management of large and complex operations, and a two-day conference was held at Airlie House for an intensive examination of major program areas of the Institute with a view to expanding or realigning them as basic components of an expanded effort. Two conferences were also arranged to seek the advice of outside consultants. Six eminent scientists in the cancer field met for an evening and a full day with NIH and NCI senior staff people to discuss the scientific aspects of a major Federal cancer research endeavor, and a group of management experts were also brought in to consider the organizational and administrative aspects of a large scale effort.

The NCI Director was in frequent consultation with the Director, NIH, and with the Secretary and officials of the Department in the development of a plan to implement the President's plans for an enlarged cancer program. The main provisions of the draft legislation sent to Congress, as set forth in a letter from the Secretary to Rep. Carl Albert, Speaker of the House of Representatives were:

- Establishment of a Cancer Cure Program within the National Institutes of Health under a Director who would report to the President. The Program, working in coordination with the total research effort of NIH, would seek to conquer cancer at the earliest possible time.

- Establishment of a Cancer Cure Advisory Committee to advise the President, the Secretary, and the Director of the new Program on ways and means of conquering cancer. The existing National Advisory Cancer Council would be preserved, but would operate within the new framework essentially as an arm of the Advisory Committee.

- The National Cancer Institute would become a part of the Program.

Renewed interest in the cancer problem and the need for an expanded Federal cancer research effort can undoubtedly be largely ascribed to mounting public concern. The cutbacks in funding of NCI grants and contracts which became necessary in fiscal 1970 brought a strong protest from the scientific community, and as the issue became publicized thousands of individuals were motivated to write letters to the President and to their representatives in Congress demanding that more cancer research funding be provided. A large volume of these letters were referred to the National Cancer Institute, giving the Institute's public information staff an unprecedented task of responding to the inquiries. A serious backlog of unanswered mail was tackled by a temporarily augmented writing and typing staff, and by spring the situation was returned to normal.

Widespread interest in the cancer problem and proposals for increased Federal support of research in turn greatly stimulated interest on the part of the public media in all aspects of the problem. Newspapers and magazines, and particularly the television media, made exceptional demands on the Director and the Institute's program directors, scientists, and information staff for assistance in developing special articles and programs. Coverage of the spring scientific meetings also reflected the heightened interest in cancer.

Research progress was marked by a number of significant developments.

Advances in Molecular Biology

Several basic research discoveries of 1970, achieved with NCI support, offer hope for eventual understanding and control of cancers in man.

One, the first complete synthetic duplication of a gene, was announced by Dr. H. Gobind Khorana as the culmination of his 10-year NCI grant-supported studies of nucleic acids and the biological code. (With Dr. Marshall W. Nirenberg of the National Heart and Lung Institute and Dr. Robert W. Holley of the Salk Institute, Dr. Khorana won a Nobel Prize in 1968 for previous work on nucleic acid metabolism. He is now at the Massachusetts Institute of Technology.)

At the University of Wisconsin, Dr. Khorana and Dr. T. Mathai Jacob worked out a method of building the 77-nucleotide chain of the DNA yeast gene which in turn synthesizes alanine transfer RNA. If, as Dr. Robert J. Huebner of NCI has hypothesized, an inherited cancer gene is a factor in the cause of most cancers, the ability to correct genetic mistakes with the use of synthetic genes may some day be possible.

Scientists financed in part by the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, the U.S. Atomic Energy Commission, and the National Science Foundation reported a "candid camera" view of genes in action. Using the electron microscope, a team of investigators headed by Dr. Oscar L. Miller, Jr., of the Oak Ridge National Laboratory were able for the first time to observe the production of proteins--an essential life process. Viewing the electron micrographs, the scientists were able to distinguish genes, enzymes, and other components of the cells' production line for proteins. Such observations will prove useful if and when correction of genetic errors associated with human cancer and other diseases comes within reach.

Other NCI-supported scientists at the University of Wisconsin, Drs. Howard M. Temin and Satoshi M.Mizutani,startled the scientific world with findings published in Nature magazine that seemed a reversal of a central dogma of molecular biology. Until that time ribonucleic acid (RNA) was considered a servant chemical in the cell, usually formed from a DNA blueprint to carry out instructions coded in the DNA. Instead, said Drs. Temin and Mizutani of the University of Wisconsin's McArdle Laboratory for Cancer Research, in some instances at least, RNA is the blueprint from which DNA is formed, and in those instances controls the cell's heredity.

The seeming reversal of conventional information transfer occurred in a cancer-causing RNA virus, Rauscher leukemia virus of mice.

In the same issue of Nature (June 27, 1970), Dr. David Baltimore, of MIT, supported by NIAID as well as NCI, reported the presence of the new-found enzyme in the Rauscher virus that causes leukemia in mice as well as in the Rous virus of chickens.

In August, Dr. Sol Spiegelman of Columbia University, working with NCI's Special Virus Cancer Program, also confirmed the existence of the enzyme and its importance to cancer research. He reported the presence of the RNA-dependent DNA polymerase in seven RNA viruses associated with tumors of mice, cats, monkeys, and birds.

Virus-Cancer Research

In November 1970, Dr. Robert C. Gallo of NCI's Human Tumor Cell Biology Branch, with colleagues at Litton/Bionetics Research Laboratories under contract to NCI, reported finding the RNA-dependent DNA polymerase enzyme in white blood cells from 3 patients with acute leukemia. The enzyme was not detected in white blood cells from 48 normal blood donors. Because this enzyme seems always present in RNA tumor viruses of animals, its discovery in cells from leukemia patients is considered good supportive evidence that viruses are associated with cancers in man. Moreover, Dr. Gallo reported that in test-tube experiments with white blood cells from leukemia patients, an antibiotic called n-demethyl rifampicin was able to inhibit completely the enzyme's activity. Previously Dr. Maurice Green of St. Louis University, with contract support from NCI's Special Virus Cancer Program, had discovered that the compound was able to inhibit the enzyme in cells from animals.

In September, Dr. Robert J. Huebner and his colleagues proposed in the Proceedings of the National Academy of Sciences that an inherited cancer gene present even before birth could also be a growth factor in the developing embryo. The inherited genetic material, which is common to a group of RNA viruses that cause cancers in mice, chickens, hamsters and cats, was detected through its "footprints" in healthy embryos of both laboratory-bred and wild mice.

Two other NIH scientists made a finding that offers support for the "oncogene" theory. Drs. Robert Stern of NIDR and Robert M. Friedman of NCI reported that double-stranded RNA, previously believed unique to viruses, is present in apparently normal uninfected cells as well as in infected cells.

NCI's Drs. Alfred Hellman and Arnold K. Fowler reported in May 1971, on experiments in mice which demonstrate that the postulated cancer-associated gene, the "oncogene," can be detected in certain tissues through hormone administration at near normal levels. This is made evident by the presence of the group-specific (gs) antigen associated with C-type RNA tumor virus.

The oncogene theory of the origin of cancer was first proposed in 1969 by Drs. Robert J. Huebner and George Todaro of the Institute. It rests on the premise that the gs antigen is present in all normal cells from the

beginning of life, even though inactive as a cancer inducer during most of it. It assumes that the viral genetic information is transmitted vertically at conception, from parent cell to daughter cell, rather than horizontally, from neighbor to neighbor, as in most infectious diseases. According to the theory, the potential cancer gene awaits "triggering" to cancerous growth by hormonal changes, a virus, chemical irritants, or the aging process.

In October 1970, a team led by Drs. Roscoe O. Brady of the National Institute of Neurological Diseases and Stroke, and Peter T. Mora of the National Cancer Institute described cellular effects of two DNA viruses, the simian virus 40 and polyoma virus, both of which cause tumors in rodents. The team discovered that cells transformed by these viruses have a greatly reduced amount, if any, of an enzyme called amino sugar transferase. Lack of this key enzyme prevents the synthesis of gangliosides, complex fat-like substances that normally are concentrated in cell walls. In the virus-transformed cells, lack of ganglioside synthesis coincides with a profound alteration in the surface properties of the cells, permitting them to grow against each other, pile up and overrun one another, with disregard for the normal behavior pattern called "contact inhibition," or the cessation of growth when cells pump into each other.

Type 2 herpes viruses, which had earlier been associated with cervical cancer, were further implicated by a report published in the September 1970, issue of the Journal of the National Cancer Institute. Dr. Laure Aurelian and her colleagues at The Johns Hopkins School of Medicine presented immunologic evidence that the virus was present in cells obtained from 95 percent of women with very early, pre-cancerous "atypical" cells of the cervix studied, as well as in 100 percent of the cells from women with advanced, invasive cancer of the cervix.

Chemical Causes of Cancer

The publication of the National Advisory Cancer Council's Progress Against Cancer, prepared with the assistance of the NCI staff, was a highlight of the year. Its theme was "Research on Chemical Carcinogenesis." PAC '70 describes various types of chemical pollution known to cause cancer: environmental agents, occupational chemicals, and carcinogens in air, food, drugs, and especially cigarette smoke. It pinpointed cigarette smoking as a "private pollutant," citing more than 60,000 deaths a year in the United States from an "epidemic" of lung cancer attributed mainly to this habit. In the 100-page publication, the National Advisory Cancer Council spelled out the new Federal restriction on cigarette advertising, which went into effect at the beginning of 1971.

A NCI publication called Carcinogenesis Abstracts was issued regularly during 1970 to bring together information about work being done in all fields of carcinogenesis and encourage interdisciplinary communication. Accordingly, Carcinogenesis Abstracts provides a central source for accurate summaries of carcinogenesis literature published throughout the world.

It is expected that three additional supplements to Survey of Compounds Which Have Been Tested for Carcinogenic Activity, which is compiled by the NCI, will be published starting late in 1971. These will follow Supplement 2, which covered the years 1954 through 1960.

To improve the efficiency of research for the identification of cancer-causing substances in the human environment, in November 1970 the NCI initiated a special study at the Stanford Research Institute of Menlo Park, California. The study was designed to establish priorities for screening in laboratory animals of the countless new compounds introduced into the environment each year, in addition to those already in existence. Researchers are engaging in a broad assessment of the environment. Under contract, industrial chemicals, foodstuffs, air and water pollutants are among the thousands of materials being considered.

The NCI continues to test and retest environmental chemical pollutants, including agricultural chemicals such as pesticides, artificial sweeteners such as cyclamates and saccharin, food preservatives, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Substitutes for those compounds found to be carcinogenic are also being tested.

Scientists continue to investigate the cancer-causing potential of nitrosamines, a class of compounds known to induce tumors in many animal models. As there is no evidence that man is immune to their action, nitrosamines are being tested alone and in combination with other compounds. Sometimes nitrosamines are formed when foodstuffs combine with chemicals in the digestive system. Others are preformed, as in tobacco smoke, grains, and alcoholic beverages, where they are present in concentrations of less than five parts per million. Higher concentrations of nitrosamines have been found in some nitrite-preserved fish meal intended for animal feeds.

The Tobacco Working Group, an offshoot of the National Cancer Institute's Lung Cancer Task Force composed of scientists and physicians from the Government and universities, and representatives from the tobacco industry and other institutions, is in the process of aiding in the development of a less hazardous cigarette. The group is concentrating on the identification and development of meaningful laboratory assay systems to guide the investigator in identifying and modifying the harmful constituents of cigarette tobacco and smoke. It hopes to clarify further the relationships between smoking and health. Under a number of contracts totaling more than $1 million, studies are going forward, including assays of various modifications of cigarette smoke and its effects on animal systems, and the role of particulate material in the production of respiratory cancers.

Immunologic Approaches to Cancer

In a December 1970 issue of the journal Science, four scientists--Drs. Berton Zbar, Irwin Bernstein, Tomiko Tanaka and Herbert J. Rapp--reported an immunizing technique utilizing a vaccine against tuberculosis called BCG. In their research, tumor cells obtained from guinea pigs were mixed with TB

vaccine, and the mixture was injected into the skin of unimmunized guinea pigs. An inflammatory response to BCG occurred, and there was no progressive tumor growth. Without BCG, the tumors grew progressively, spread through the animals' bodies, and killed them. The scientists conclude that improved immunity to tumors may be possible by mixing some of an individual's own tumor cells with BCG and then reinoculating them into the individual.

Scientists supported by the National Cancer Institute and the National Institute of Allergy and Infectious Diseases demonstrated a relationship between interferon levels in the skin of patients and the outcome of varicella infections such as shingles and chickenpox. Patients with lymphomas or leukemias, and those receiving immunosuppressive therapy, are known to be unusually susceptible to these infections; they are also likely to have serious complications from smallpox vaccination. The investigators speculated that the ability to produce high levels of cutaneous interferon may be related to white-cell function which was noticeably impaired in those cancer patients with the more serious or fatal varicella infections.

New immunological evidence reported in April 1971 by Institute scientists supports the theory that there is an immune response to antigens on leukemia cells. The research by Dr. Eugene B. Rosenberg and colleagues Drs. Ronald B. Herberman, Paul H. Levine, and John R. Wunderlich was a study of cellular reactions to cells of leukemia patients and their normal identical twins, by cells of family members and unrelated normal individuals.

Previous studies using mixed lymphocyte culture and skin tests have indicated that patients with leukemia may have an immune response to tumor associated antigens. The study by Dr. Rosenberg and his colleagues extends these observations. Identical twins were selected for participation in this study to minimize the possibility that the observed reactions were due to normal tissue differences between participants.

Six sets of identical twins were included in the present research into cell cytotoxicity. In four of six families studied, lymphocytes from family members reacted against lymphoid cells from the patient, but not from the identical twin. Lymphocytes from unrelated normal individuals also reacted against cells from two patients. One of five fathers, two of five mothers, four of seven brothers, one of seven sisters, and four of seven normal unrelated individuals gave positive lymphocyte cytotoxicity reactions to the leukemia cells. When cells from family members and normal controls were tested against cells from the normal twins, no positive reactions resulted.

The investigators reported that cells from adults over 21 who were included as normal participants in the study showed a greater tendency to react than cells from less mature participants. They concluded that distinctive antigens may be present on leukemia patients' cells and indicated that positive cellular cytotoxicity reactions to these antigens by family members and normal unrelated individuals suggest previous sensitization, possibly by a virus.

Control of virus-induced cancers in man seems to be heading toward eventual success. Although some of the pathways are changing from conventional vaccines to newer immunologic and preventive measures still in the developmental

stage, the National Cancer Institute's Dr. Frank J. Rauscher, Jr. said at the World Health Organization's International Conference on the Application of Vaccines against Viral, Rickettsial, and Bacterial Diseases of Man in December 1970.

In a chart showing progress toward control, Dr. Rauscher pointed out that in only seven years, scientists have come to the "proof of cause" state for herpes-type DNA viruses in Burkitt's lymphoma and are nearing control. The C-type RNA viruses, in only two years since their detection in human sarcomas, have reached an advanced study stage and scientists are near control of these same viruses in laboratory animals.

Dr. Rauscher said that the new measures for possible control might include immunization involving virus-induced transplantation antigens, substances on the surface of cells that have been exposed to a virus and stimulate antibody production against that virus or against their induced cancer cells. Another method might be interference with the action of an important enzyme, the RNA-dependent DNA polymerase found to be associated with RNA virus-caused cancers in animals and in white blood cells from leukemia patients. Still another measure might be the use of natural or synthetic repressors for viral activity, an avenue of inquiry suggested by Dr. Robert J. Huebner of the National Cancer Institute as the next step in cancer research to follow his "oncogene" theory. Or it might be interference with the environmental or hormonal derepressors which Dr. Huebner believes may "trigger" these latent but naturally occurring virus genes in all vertebrates, man included. However, a "standard," or conventional type, vaccine may still be a practical approach to some human cancers, said Dr. Rauscher; he noted that cancers in mice and chickens can be greatly reduced by the use of standard virus vaccines.

In April 1971 new evidence reported by National Cancer Institute scientists suggested that advanced cancer patients, like animals with advanced cancer, may have an immune deficiency that prevents them from using transferred immunity. The findings by Dr. Irwin D. Bernstein and colleagues Drs. Daniel E. Thor and Herbert J. Rapp upset traditional thinking about cancer immunotherapy. Early efforts now under way to treat cancer by passive transfer of immunity are based on the assumption that the cancer patient is capable of using the transferred immunity. The new NCI finding suggests that, in addition to the transferred immunity, the presence of mononuclear cells is required for anti-tumor effect. Present findings indicate that these nonspecific infection-fighting mononuclear cells (macrophages) are absent or impaired in patients with advanced cancer. The studies show that when animals have advanced cancer, transfer of immunity is not effective. But in animals without cancer, immunity can be transferred and inhibits growth of tumor cells when they are injected. Dr. Bernstein and his colleagues attribute the ineffective transfer of immunity in tumor-bearing animals to a lack of or deficiency in mononuclear cells, large non-specific infection-fighting cells, commonly called macrophages.

Chemotherapy

During calendar year 1970 some 15,000 compounds, including 8,500 synthetic chemicals and more than 6,000 natural products from fermentation, plant and animal sources, were tested by the National Cancer Institute as potential anticancer agents. The compounds were tested against mouse leukemia L-1210, a tumor model found to be the most reliable system for predicting the response of human cancer to drug therapy. In addition, some of the natural products were given a second test, this time against tumor P-388, a mouse leukemia similar to L-1210 but able to detect the activity of smaller quantities of crude material.

As a result of such initial screening against rodent cancer, six materials showed sufficient antitumor activity to be selected by NCI scientists for pharmacology-toxicology studies in large animals. Such studies assure that the test compounds will be non-toxic and safe to use, and furthermore will help to determine the starting dose for human drug administration. Six compounds passed the National Cancer Institute's stringent tests in animals for pharmacologic evidence of activity and safety, and were accepted for study of human cancer. The Institute filed Investigational New Drug applications with the Food and Drug Administration for all six. Then the painstaking, long-term clinical studies were begun to determine whether or not the materials will offer cancer patients therapeutic benefits not now available through conventional methods of treatment.

A National Cancer Institute scientist reported on test-tube experiments in which a derivative of an antibiotic called rifampicin was able to inhibit completely an enzyme found in the white blood cells of leukemia patients. The enzyme, called RNA-dependent DNA polymerase, had been reported earlier in the year to be associated with certain viruses that cause cancer in animals. In laboratory experiments with human leukemia cells, the National Cancer Institute investigator, Dr. Robert C. Gallo and his colleagues found that the antibiotic rifampicin was able to block the enzyme's activity only to a small degree. A much more effective related compound, n-demethyl rifampicin, and other closely related compounds will be evaluated by NCI scientists for possible effectiveness against cancers in animals. If found active against animal cancers, they will then have to be demonstrated to be relatively non-toxic before they can be evaluated against leukemia or other forms of human cancer.

At a conference in November, attended by physicians from 20 States and the District of Columbia, research results with two anticancer drugs (ortho para'-DDD and mithramycin) were described. Ortho para'-DDD, a drug related to the insecticide DDT, was reported as helpful in some patients with advanced cancer of the adrenal cortex. Also described was its usefulness in some cases of Cushing's disease, a rare disease of abnormal growth characteristics sometimes due to an adrenal tumor.

At the same conference the usefulness of the antibiotic mithramycin in some cases of cancer of the testes was explored. The drug is also of benefit in some patients with Paget's disease of bone and in problems of calcium imbalance.

In August 1970, National Cancer Institute scientists reported that a 4-drug treatment developed in 1964 for use in advanced Hodgkin's disease is more than doubling the survival time of patients who respond to treatment. Thirty-five of 43 patients (81 percent) treated at the beginning of the study with the four drugs (vincristine, procarbazine, prednisone, and an alkylating agent) responded with a complete remission. This is four times the rate of complete remission usually achieved in advanced Hodgkin's disease.

In November 1970, Drs. Max M. Burger and Kenneth D. Noonan of Princeton University, working with National Cancer Institute grant support, reported in the journal Nature on experiments with trypsinized Concanavalin A (Con A), a protein obtained from the jackbean plant. The NCI-grantee scientists found that this protein agglutinates, adheres to and clumps malignant cells of the mouse, growing in tissue culture. By so doing, it covers up an exposed layer of surface receptors on these cells, mimicking the surface pattern of normal cells and restoring their growth pattern to the normal state of "contact inhibition." Con A's effectiveness in returning malignant cells to a normal growth pattern continues until the cover layer is removed. These findings have aroused the interest of National Cancer Institute scientists engaged in chemotherapy research, and during 1971 they will evaluate Concanavalin A's effects on the growth of cancer in animals.

Scientists of the Institute and the Veterans Administration evaluated the drug diethylstilbesterol in patients with advanced cancer of the prostate, and their studies suggested that a 1.0 mg. daily dose may be as effective as 5.0 mg.in controlling prostatic cancer, but without the associated risk of cardiovascular death. On the basis of these results, the scientists concluded that the 5.0 mg. dose of diethylstilbesterol is no longer justified as initial therapy in stages III and IV of prostatic cancer.

Institute scientists reported in April 1971, that in developing a new therapy for advanced stages of chronic granulocytic (myelocytic) leukemia, they have demonstrated a possible means of predicting the response of patients to this treatment. Drs. George P. Canellos, Vincent T. DeVita, Jr., Jacqueline Whang-Peng, and Paul Carbone reported on remissions achieved with a combination of two drugs, vincristine and prednisone. This therapy has not been previously used in the "blastic" phase of chronic myelocytic leukemia, a type of leukemia in which there is an overproduction of granulocytes. Dr. Canellos and his associates studied a series of 30 consecutive patients at the Clinical Center of the National Institutes of Health, Bethesda, Maryland as they reached the advanced, "blastic" stage of the disease, characterized by an excess of immature cells. The drugs vincristine and prednisone, administered intensively for at least two weeks, produced complete remissions (temporary disappearance of all evidence of disease) in 6 of the 30 patients. Three patients achieved partial remissions. Another 8 patients showed some antileukemic effects from the therapy. In 13 patients there was no benefit from the combination of drugs. The median survival in the remission group of nine patients was ten and a half months (ranging from three to 28 months) following treatment. Two of these patients are still alive. Among the nonresponders, none of whom is now alive, there was a median survival of two and a half months.

Another report presented in April 1971 indicated that a 3-drug combination treatment developed at the NCI for advanced lymphosarcoma (cancer of the lymph system) is achieving a higher survival rate than treatment programs previously reported. Although early stages of this cancer are usually best managed with radiation therapy, the advanced disease is usually treated with single drugs of many kinds, one at a time. However, with single-drug treatment, it was not possible to eradicate all evidence of the cancer in patients with advanced lymphosarcoma, and more than half of such patients die in two years or less.

In January 1967 a new drug treatment for advanced lymphosarcoma was initiated by a research group at the Solid Tumor Service, Medicine Branch of the National Cancer Institute. Drs. Charles M. Bagley, Vincent T. DeVita and George P. Canellos reported their findings this year. Three drugs, cyclophosphamide, vincristine, and prednisone, were given in large doses in intensive 5-day courses in an attempt to obtain a high rate of complete remissions and of prolonged survivals. Thirty-five patients were included in the study, 22 of whom were men and 13 women, with a median age of 45. Only 2 patients had been previously treated by radiation; none had been previously treated by anti-cancer drugs. The lymphosarcoma of all 35 patients, Dr.Bagley said, was in such an advanced stage that none could have been cured by radiation. At the end of the 3-drug therapy, 20 patients (57%) had complete remissions of lymphosarcoma--that is, they showed no evidence of cancer by any test or biopsy. Twelve patients (34%) had partial remissions, and 3, (9%), failed to show any response. Of the 20 patients who achieved complete remissions, 18 remained in complete remission 1 year later.

In December 1970 the Institute's Clinical Investigations Branch sponsored a two-day meeting for clinicians who cooperate in the evaluation of anticancer drugs and surgical and radiation regimens of cancer therapy at 250 medical centers. More than 125 scientists who cooperate in NCI-supported clinical investigations participated in the symposium, which was organized to improve the design of prospective randomized controlled studies of cancer therapy. Chemotherapists, surgeons, radiotherapists and biometricians participated.

In October 1970 the Institute was one of several organizations participating in a 2-day International Conference on Adverse Reporting Systems organized by the Drug Research Board of the National Research Council. Methods of detecting, evaluating, and reporting adverse reactions to drugs were discussed. The Drug Research Board had found that in the United States detection and scientific evaluation of adverse reaction to drugs were deficient both in technology and reporting systems. The Board concluded that much current data on adverse reactions were of questionable value, and many programs were developing information that did not effectively reach concerned investigators. NCI's Associate Scientific Director for Experimental Therapeutics took part in a session devoted to "Coordination of Programs."

During the year the Institute continued to take advantage of technology available from the National Aeronautics and Space Administration by collaborating with NASA scientists and engineers to help remove certain technological obstacles to NCI's Clinical Trials research. NCI collaboration with the

Biomedical Application Teams (BATEAMS) of the Research Triangle Institute in North Carolina began with the analysis of a series of seven technology-oriented problems. NASA proposals now hold promise of solving five. The sixth was worked out by an NCI investigator himself; and the last is as yet unsolved. NASA data banks of approximately 500,000 items are used in one aspect of the efforts to solve problems.

Other Research

Institute surgeons, since 1954, have performed 162 pelvic exenterations for carcinoma of the uterine cervix in patients ranging in ages from 25 to 74. This surgical procedure was the initial form of treatment for 68 women with large, previously untreated cancers not amenable to lesser surgery. An actuarial five-year survival of 48.5 percent was achieved for this group. The remaining 94 patients, treated for recurrent cancer after previous radiotherapy, achieved a five-year survival of 28 percent. The total cumulative survival was 38 percent. When guidelines for patient selection are followed, the operation can now be performed by experienced surgeons with considerably less morbidity than previously observed. The report was published in the journal Cancer.

NCI scientists reported no increased incidence of fetal mortality, congenital abnormality, or complicated pregnancy among women previously treated by chemotherapy for gestational trophoblastic cancers. Previous studies had established that many cancer drugs produce malformations in the offspring of animals and humans receiving them early in pregnancy. This retrospective study evaluated the effect of cancer drugs on oocytes and possible hazards to mother and fetus in subsequent pregnancies. The reproductive performance of all patients treated successfully for gestational trophoblastic neoplasms at the NIH Clinical Center between 1956 and 1969 was studied. Fifty of the 91 women studied subsequently conceived and had 88 pregnancies which produced 71 live full-term infants (81 percent). The study was reported in September 1970.

NCI scientists meeting at a San Diego conference this year presented epidemiologic and pathologic reports on cancer of the colon and rectum--the second leading cause of cancer deaths in the U.S. Dr. John H. Weisburger, Head of the Institute's Carcinogen Screening Section, discussed the ingestion of a carcinogen absorbed and metabolized by the liver, secreted in bile as a conjugate, and the active agent liberated by bacterial enzymes in the colon. Dr. Harold H. Stewart, Consultant to the Director, NCI, discussing "Geographic Pathology of Cancer of the Colon and Rectum," reviewed the geographic distribution of these cancers in man, and described their experimental induction in laboratory animals. NCI Director Dr. Carl G. Baker addressed the conference, stressing the need for invention as well as discovery in cancer investigations, as new research approaches to the control of large-bowel cancers become available.

Information and Education

Events and issues involving Institute programs generated lively public interest throughout the year. As stated earlier in this report, the one item causing the most concern voiced through both the news media and public inquiries--was the proposal that cancer research be taken out of the National Institutes of Health and placed in an independent National Cancer Authority directly responsible to the President.

This proposal gave rise to a flood of inquiries on the part of not only the news media, but also the general public. Both the printed and electronic news media kept the Institute's public information staff busy formulating replies to questions and recommendations. The ABC and NBC television networks, and Fortune and Newsweek magazines put top-staff reporters and commentators on the story. Meanwhile, the Branch continued to give increased visibility to the Institute through the usual channels: news contact, public inquiries, and the issuance of publications.

Reorganization Items

In the Chemotherapy area, a Drug Metabolism Section was established in the Laboratory of Chemical Pharmacology.

The name of the Cancer Chemotherapy National Service Center, Chemotherapy, has been changed to the Office of the Associate Director for Drug Research and Development, Chemotherapy.

There was established a Viral and Molecular Pathology Section within the Laboratory of Molecular Biology, General Laboratories, and Clinics. The Laboratory now consists of the Office of the Chief, the Biochemical and Genetics Section, and the newly-added section.

There was established, within the General Laboratories and Clinics area, a Laboratory of Theoretical Biology, consisting of a Molecular Genetics Section, a Mathematical Biology Section, and a Computational Methods Section, in addition to the Office of the Chief of the Laboratory.

The Program Analysis Branch was transferred within the Chemotherapy Area, from the Office of Scientific Director for Chemotherapy to the Cancer Therapy Evaluation Branch. Types of appointment, position, grade, occupation code, and salary remained unchanged. In this reorganization, the functions of the Laboratory and Clinical Trials Section were assumed by the Data Processing Section. The Laboratory and Clinical Trials Section was abolished.

In the Etiology Area of the Institute, several organizational changes were effected, including the establishment of three offices within the Office of the Associate Scientific Director for Viral Oncology and redefinition of functions in the existing branches.

The following changes were made:

Office of the Scientific Director for Etiology

The Scientific Coordinator in Viral Oncology was transferred from the Office of the Associate Scientific Director for Viral Oncology to the Office of the Scientific Director for Etiology. The functional statement remained unchanged. The Office of the Scientific Director for Etiology now consists of:

Immediate Office of the Scientific Director
Administrative Management Section
Scientific Coordinator in Viral Oncology
Office of the Associate Scientific Director for Program

Office of Associate Scientific Director for Viral Oncology

The following Sections of this Office on Biohazards Control & Containment, Program Analysis & Communications, Program Resources & Logistics, and Research Support were abolished.

In the newly created Office of Biohazards and Environmental Control, there were established the Biohazards Research and Environmental Control Sections. The Virus Studies Section was transferred from the Viral Biology Branch to the new Office of the Coordinator for Ultrastructural Studies.

Viral Biology Branch

In the Viral Biology Branch there were established Experimental Pathology Human Tumor Studies Sections. The Virus and Disease Modification Section was transferred from the Viral Leukemia and Lymphoma Branch to the Viral Biology Branch.

Viral Carcinogenesis Branch

In the Viral Carcinogenesis Branch there were established the Ecology and Epizoology and Viral Genetics Sections. The Ecology and Epidemiology Section was abolished.

Viral Leukemia and Lymphoma Branch

In the Viral Leukemia and Lymphoma Branch there were established the Genetics, Tumor Virus and Viral Pathology Sections.

The Molecular Biology Section was transferred from the Viral Carcinogenesis Branch to the Viral Leukemia and Lymphoma Branch.

The following Sections were abolished:

Molecular Virology Section
Ultrastructural Studies Section
Viral Leukemia Section

Also effected was the transfer of the Epidemiologic Pathology Unit from the Office of the Associate Scientific Director for Demography, Etiology,NCI, to the Office of the Associate Director for Carcinogenesis, NCI. The Office of the Associate Scientific Director for Carcinogenesis now includes:

Epidemiological Pathology Unit
Lung Cancer Unit
Program and Data Analysis Unit

The functional statement for the Epidemiologic Pathology Unit has been amended to read: "Provides liaison necessary for the development of laboratory studies in carcinogenesis, relevant to epidemiological findings of the Migrant Project and other Demography programs. Serves as the primary reference point in the Carcinogenesis program for development of relevant studies. Serves as the Etiology focal point for the development of a colon cancer program."

Staff Changes, Activities, Honors and Awards

Dr. Carl G. Baker, formerly Acting Director of the Institute, was appointed Director.

Dr.Frank J. Rauscher, Jr., formerly Acting Scientific Director for Etiology, was named Scientific Director for Etiology.

Mr. Calvin B. Baldwin, Jr., was transferred from the National Institute of Child Health and Human Development and designated Executive Officer, NCI.

Dr. John B. Moloney, formerly Acting Scientific Director for Viral Oncology, was named Scientific Director.

Dr. Elizabeth Weisburger, of the Biology Branch, was named Assistant Editor-in-Chief of the Journal of the National Cancer Institute. A member of the Editorial Board of the Journal, Dr. Weisburger is the first to hold this new position.

Dr. Vincent T. Oliverio was appointed Acting Associate Scientific Director for Experimental Therapeutics.

Dr. Tibor Borsos was named Associate Chief of the Biology Branch, Etiology.

Dr. Ira H. Pastan was designated Chief of the Laboratory of Molecular Biology.

Dr. William D. Terry was appointed Assistant Chief of the Immunology Branch, General Laboratories and Clinics.

Dr. Michael D. Walker was named Deputy Chief of the Baltimore Cancer Research Center, NCI.

Dr. Lloyd W. Law was designated Chief of the Laboratory of Cell Biology.

Dr. Paul Carbone was elected Chairman of the Eastern Cooperative Oncology Group.

Dr. Robert J. Huebner, Chief of the Viral Carcinogenesis Branch, was given the 1970 Rockefeller Public Service Award in the category of Science, Technology, and Engineering, and shared the Kimble Methodology Award for 1970.

William M. Haenszel, Chief of the Biometry Branch, received the sixth honorary doctoral degree awarded in the history of the Universidad del Valle, Cali, Colombia.

Dr. George J. Todaro was named one of the Jaycees' "Ten Outstanding Young Men of the Year" by the U.S. Junior Chamber of Commerce.

Dr. Harry V. Gelboin received the Claude Bernard Award and was also the honorary lecturer at the Annual Meeting of the Radiological Society of North America.

Mr. H. Richard Miller, Administrative Officer, Financial Management Branch, was cited for superior work performance.

Drs. Julius White, Chief of the Laboratory of Physiology, and G. Howard Gowen, Director of Clinical Programs Centers, Special Programs Branch, retired.

Program Activities Report
Fiscal Year 1970-71
Editorial Office of the National Cancer Institute

The Board of Editors reviewed 1,299 items for publication in various journals. Those not destined for the Journal of the National Cancer Institute constituted 660 manuscripts and 244 abstracts and book reviews.

Of 395 manuscripts submitted for publication in the Journal of the National Cancer Institute, 72 were by NCI authors: 48 accepted; 3 rejected; and 21 pending. Authors from other research institutions submitted 323: 158 accepted; 59 rejected; and 106 pending. Of these, 118 were received from other countries.

Volumes 44 and 45 of JNCI totaled 2,666 double-column pages.

One NCI Monograph totaling 594 pages was published: #33 Patterns in Cancer Mortality in the United States: 1950-1967.

ANNUAL REPORT
OFFICE OF THE ASSOCIATE DIRECTOR FOR PROGRAM PLANNING AND ANALYSIS (OADPPA)
NATIONAL CANCER INSTITUTE
July 1, 1970 - June 30, 1971

Office of the Associate Director

The planning and analysis activities performed by the staff of OADPPA fall into two categories: the formulation of program and operational plans for new research efforts; and the revision and updating of ongoing programs based on research results. The approach to planning as a dynamic process, and not single discrete activities spaced in time, provides the planning staff with a continual base for relating and working with the scientists and managers in the operating programs. All planning activities involving NCI programs are conducted usually at the request of the program area and by joint planning teams consisting of OADPPA and program area staff. The practice of assigning a member of the OADPPA staff to "live" with the program area has continued to be an effective and mutually productive arrangement.

During FY 1970 the excitement and promise of an expanded National Cancer Conquest Program called for by the President has engaged the planning staff and senior program operators in the initial phases of formulating an overall strategic operating plan for the Cancer Conquest Program. These initial activities will be the basis for engaging large numbers of scientists and managers across the country in the detailed development of a national plan.

Dr. Robert Greenfield, Chief of the Program Analysis and Formulation Branch left OADPPA to accept the position of Associate Director for Planning and Analysis at the National Institute of General Medical Sciences.

Dr. Leon Ellwein joined the OADPPA staff upon completion of his doctorate program in Industrial Engineering and Operations Research at Stanford University.

Mr. John McShulskis, a Research Director at Booz-Allen Hamilton Applied Research, joined the OADPPA staff as Chief, Systems Operations Planning Branch.

Interest in the Convergence Technique (developed at NCI) as a method for research planning continued to be expressed by other Federal Agencies and non-Federal organizations. Mr. Carrese was invited to give several presentations on the method. Of particular interest was the invitation by the Deutsche Forschungemeinshaft (German Research Council) for Mr. Carrese to hold a two-day working seminar on methods for biomedical research planning in Hanover, West Germany.

Publications:

The Convergence Technique - An Alternative Strategy for Programmatic Research and Development, presented at the 64th Annual Convention of the American Vocational Association, New Orleans, Louisiana, to be published in the Monograph of the Convention.

ACTIVITIES OF THE PROGRAM ANALYSIS AND FORMULATION BRANCH (PAFB)

This Branch is concerned primarily with substantive matters in cancer research and in related matters. It prepares position papers outlining alternative approaches, guidelines and procedures and devises models for the review, evaluation and implementation of NCI programs. The Branch, including Drs. Greenfield, Cantarow, Klein, participates in discussions and prepares reports and documents on progress towards major objectives and identified needs of the Institute. These discussions include in-depth analysis of current programs, initiation of major program modifications and the development of outlines for new programs. An integral part of the Branch's operations concerns identification, review and analysis of resource requirements for NCI programs and the development of plans, approaches and guidelines contributing to their implementation.

The members of the Branch provide assistance to the Director, the Scientific Directorate, the National Advisory Cancer Council and the Chiefs of the different line areas of the Institute on matters relating to research planning as described. Some examples include: the development of structures and guidelines for formulating new research programs or for amending existing ones, development of logic arrays for new programs, review of manuscripts, development of responses to queries emanating outside the Institute. The Branch also maintains liaison with individuals and groups outside the NCI on matters relevant to cancer.

Specific activities engaged in by the Branch during the past year are provided in the paragraphs that follow.

I. Development of National Programs on Organ-Type Neoplasms

Drs. Greenfield, Klein and Cantarow participated in the formulation and development of preliminary plans and guidelines for projected programs on cancers of the colon and urinary tract and of organ-site neoplasms generally.

A. National Program on Cancer of the Large Bowel

Through the joint efforts of Mr. Carrese, Dr. Greenfield (PAFB) and Dr. Burdette of M.D. Anderson Hospital (MDAH-Houston), a general logic flow diagram was prepared for adenocarcinoma of the colon. This was presented to the National Advisory Cancer Council during the meetings of November 1970. Through continued discussions and meetings of members of PAFB with investigators and research administrators concerned with cancer of the large bowel, an agreement was prepared between MDAH and the NCI on establishment of a National Program on Cancer of the Large Bowel to be managed by a Program Director and Headquarters Staff at MDAH and supported by the grant mechanism. The agreement included a description of the responsibilities of a management group, operations of a reviewing group for grant proposals submitted to the Program, the role of the MDAH in supporting the Program and the relationship of the Program to the N.A.C.C.

B. National Program on Urologic Cancer

Drs. Klein and Greenfield served as consultants to the Pre-Task Force on Cancer of the Bladder-Prostate, under the chairmanship of Dr. R. Flocks, and aided in the development of preliminary plans and approaches to the formation of a national program on urologic cancer. The staff members of PAFB also assisted the Pre-Task Force in developing a framework for a logic diagram for cancer of the bladder. Dr. Klein prepared a detailed survey of the distribution of effort (by state, city, institution, investigator) for cancer of the urinary bladder, prostate and kidney to identify possible sites for a management focus for a national program and active investigators in each field. Level of research support for these fields of cancer by the American Cancer Society also was secured by the Branch.

C. National Program on Breast Cancer

Dr. Greenfield (PAFB) participated in the deliberations of the NCI Working Group on Breast Cancer as this Group discussed plans for developing a National Program for this neoplasm and a major activity, in the same, intramurally at NCI. The Working Group is collaborating with the Breast Cancer Task Force in developing this National Program.

D. Development of a General Logic Framework Applicable to all Programs on Organ-Type Neoplasms

Drs. Cantarow and Klein of the PAFB in conjunction with Dr. Ellwein (SOPB) are considering different approaches and methods for development of a general logic framework that would be applicable to any organ-type tumor program.

II. Workshops on Carcinogenesis

Dr. Abraham Cantarow served as NCI representative to the Core Group of the Workshops on Carcinogenesis being conducted under a grant to the Interdisciplinary Communications Program of the Smithsonian Institution. Two workshops were held during the past year (October 26-29, 1970; February 21-24, 1971).

III. Survey of Compounds Which have been Tested for Carcinogenic Activity

A detailed analysis and critique of PHS publication no. 149, and its supplements, entitled Compounds Which have been Tested for Carcinogenic Activity was prepared by Dr. Klein at the request of the Director. The Analysis identified the strengths and weaknesses of the surveys and provided suggestions for their improvement. The accumulation, storage, processing and dissemination of data on testing chemicals for carcinogenic potential will assume increased importance in the future in parallel with projected plans to enlarge current NCI supported activities in this field.

IV. Assessment of Grant Supported Research Proposals on the Basis of their Relevance to Cancer

At the request of the Director, a report was prepared by Dr. Klein on this topic. The report was concerned with the need for assessing degrees of relevance of research proposals to cancer, alternative approaches used in determining relevance, the extent to which current grant supported programs are reviewed for relevance, and factors relevant to assessing extent of relevance to cancer. A questionnaire was prepared incorporating these factors.

V. Review of NCI Task Forces

Dr. Greenfield at the request of the NACC, prepared and presented a review of NCI Task Forces, past and present, at the June 1970 meeting of the Council. This included a history of the task force concept, a review of productivity for past task forces, and the present status of task forces in the Institute.

VI. Immunology-Immunotherapy Study Group

The Immunology-Immunotherapy Study Group of the NCI under the chairmanship of Dr. Law was assigned by the Scientific Directorate the task of reviewing and assessing the status of immunologic research within the NCI and of identifying leads or scientific opportunities that should be pursued with more vigor than now. Dr. Klein of PAFB is a member of this Study Group which meets weekly. The Group is currently preparing a report for the Scientific Directorate and the Director.

Honors:

Dr. Abraham Cantarow: Member of the Board of Trustees, Jefferson Medical College
Member of the Policy Committee of the American Association for Cancer Research
Member of the Program Committee of the Seventh National Cancer Congress

Publications:

Cantarow, A.: Responsibilities of the association in a changing world: presidential address. Cancer Research 30: 1913-1917, 1970.

ACTIVITIES OF THE SYSTEMS AND OPERATIONS PLANNING BRANCH (SOPB)

SOPB has continued in its association with various areas of the Institute by providing general management consultation in the development and application of systems and operations planning techniques. The major efforts have been directed toward providing support to the targeted research efforts. The specific areas of activity with which the SOPB has been associated include:

I. Support in the Analysis and Revision of Existing Program Plans

As research programs progress and new developments surface, the existing program plans must be reviewed and updated or completely revised to reflect these changes. This is a continuing activity which has resulted in a significant effort in two major program areas:

Periodic meetings are being carried out with the Special Virus Cancer Program (SVCP) Management to formulate a revised program plan reflecting the current key research problems, program decisions, and decision criteria. Specifically, recent developments and new leads provided by the oncogene hypothesis of Drs. Huebner and Todaro are being integrated into the program plan. The convergence technique developed by Mr. Carrese and Dr. Baker continues to be the method used for structuring the research logic. It is anticipated that these planning efforts will be expanded to consideration of problems related to program monitoring and control.

SOPB has begun working with members of the Chemical Carcinogenesis Program Staff, Etiology Area, to review current research efforts and program plans. The segment of the program to which each contract contributes has been identified and efforts are being directed towards identifying the specific kinds of data and information required to satisfy the program decision criteria. This effort is likely to continue into the next fiscal year.

II. Support in New Planning Activities

SOPB has provided staff support to the planning activities of the Breast Cancer Task Force Steering Committee. A major effort centers around the development of research plans utilizing the convergence technique for a program in breast cancer. The program is of national scope involving simultaneous and coordinated efforts in prevention, detection and diagnosis, and treatment of the disease.

III. Initiation of Efforts to Develop a Model to Simulate the Chemotherapy Drug Development Process

In a joint effort with the Systems Analysis and Planning Officer, Chemotherapy Program, SOPB has been engaged in initiating an investigation of the desirability and potential usefulness of simulating the drug development process to suggest possible actions for improving its efficiency. The simulation will seek to provide a means for a priori evaluation of potential alterations in the screening system as well as lending a better understanding of the logistical flow of compounds through the system.

IV. Joint SOPB and PAFB Activities

SOPB collaborates and consults on a continuing basis with PAFB in reviewing and analyzing various ongoing programs. Preliminary efforts are also being directed towards the development of general guidelines for use in planning organ site programs.

Publications:

Ellwein, L. B.: Fixed charge location-allocation problems with constraints on capacity and the number of sources, presented at the 38th National Meeting of the Operations Research Society of America, Detroit, October 1970.

Ellwein, L. B.: A flexible enumeration scheme for zero-one programming, presented at the 39th National Meeting of the Operations Research Society of America, Dallas, May 1971.

SCIENTIFIC AND TECHNICAL INFORMATION OFFICE (STIO)

The major types of work performed by the Scientific and Technical Information Office (STIO) of NCI during Fiscal Year 1971 are listed below:

a) STIO has continued to design, develop, and operate computerized systems for analysis, indexing, storage, retrieval, and publication of information about NCI-supported research projects. The output of these systems is used by NCI administrators engaged in program analysis and program planning activities.

b) During the past year, STIO began to design computer systems, and write computer programs for processing data and preparing reports and listings for the Budget and Personnel offices of OD, NCI, and for other groups within NCI.

c) Because of the need for rapid transfer of published research results, STIO is experimenting with computerized methods for Selective Dissemination of Information (SDI). This type of current-awareness service could supply scientists with a steady stream of useful abstracts describing publications related to their research.

d) STIO has continued to provide information about scientific and technical activities of NCI to individuals and organizations (such as the American Cancer Society, the National Science Foundation, and the Federal Council of Science and Technology) on request.

e) STIO also serves as a focal point for collecting data, answering questions, and handling special problems related to all information processing and publishing activities of NCI.

Some of the specific projects undertaken in Fiscal Year 1971 are listed below:

I. Multi-Level Analysis of Grants and Contracts supported by NCI in FY 1970

Brief but informative abstracts of every grant supported by FY 1970 funds have been prepared, and abstracts of every contract are being written. Abstracts of the 1,143 grants were grouped together, pyramid-like, to form 149 sub-categories of cancer research, 27 broader research categories, and 5

major research areas. Abstracts, fiscal data, and project identification were entered on a magnetic tape which was used to produce a hierarchical summary called Multi-Level Analysis of Research Grants by Scientific Category for Fiscal Year 1970. A similar publication dealing with contracts will be published in mid 1971.

II. Index on Experimental Cancer Pathology:

In collaboration with Dr. Harold Stewart, a computer system has been designed and implemented for producing an index to slides and other data in the Registry of Experimental Cancers. In addition to designing the system, the STIO has helped to hire and train a coder for entering data into the system. The indexes produced by this system will give easy access to experimental animal tumor data that would otherwise be virtually inaccessible.

III. Hierarchical Classification of Biomedical Information:

An automated method for producing and updating enumerative hierarchical decimal classifications of biomedical information on magnetic tape is now in the final stages of testing. A small classification in the field of information science and technology is being used as a sample data base to test the system. Previously, such classifications required extensive retyping of loose pages to keep it up-to-date, and the problem of making changes in an alphabetical index to the classification and of keeping track of cross-references was impossible. The new system has solved many of these problems by linking categories in the classification with alphabetical terms referring to the categories. Using this new system, a change in the classification triggers a change or a change-message for the alphabetical index (and also triggers a change in any cross links to or from the changed category).

IV. Computer systems have been designed for maintaining the NCI personnel roster on an on-line computer system (WYLBUR). The STIO has been active in improving the format, developing methods for automatically preparing an index to this roster, and making it possible to convert from awkward computer printouts to convenient xerox copies for use in about 30 different locations within NCI. Computer methods for easy updating of this file and for using the data to prepare special reports for the Budget and Personnel office are being developed.

V. Publications:

Schneider, John H: Indexing and selective dissemination of scientific information. Science (in press), 1971.

Schneider, John H.: Automated methods for analysis of large research and development programs, submitted for publication in Proceedings of the American Society for Information Science 9, 1971.

Research Information Branch

Office of the Director

National Cancer Institute

Program Activities Report, July 1, 1970-June 30, 1971

The public information activities of the National Cancer Institute were markedly affected by designs and developments during the year for the greatest innovation and expansion of cancer research since the establishment of the Institute in 1937. These movements in both the legislative and executive branches of the Government evoked an unprecedented expression of public interest in which NCI became heavily involved.

Events began early in 1970 with Senate approval of a resolution introduced by Senator Ralph W. Yarborough authorizing the Committee on Labor and Public Welfare to study the state of the art in cancer research and the possibilities for accelerated progress. A National Panel of Consultants on the Conquest of Cancer appointed to undertake this assignment delivered its report in December. Senator Yarborough introduced a bill in the 91st Congress to implement the Panel's recommendations, chief of which was to establish a National Cancer Authority as an independent agency which would absorb the National Cancer Institute. Similar measures were introduced in the 92nd Congress by Senator Edward M. Kennedy and Representative Claude Pepper. In his State of the Union Message to the Congress in January 1971 President Nixon requested an extra $100 million for cancer research and said he would ask later for any additional funds that could be effectively used. In his subsequent Health Message the President announced that he was directing the establishment of a Cancer Conquest Program at the National Institutes of Health to carry out his plan.

Increased public attention centered on the cancer problem by these developments was stimulated by extended coverage of the subject by the printed and electronic media including news stories, special articles, and television features. Newsweek did a cover story which appeared in the February 22 issue, and Newsweek's news feature service to newspapers in this country and abroad planned several articles. Fortune magazine assigned a member of its Board of Editors to write a comprehensive article. The ABC network sent a commentator and camera crew to NCI to interview the Director and film laboratory scenes for a program broadcast as a news feature on February 18. This coverage served, in turn, to prompt direct expression by individuals and organizations in the form of letters, petitions, and resolutions addressed to the President, members of Congress, the Secretary of Health, Education, and Welfare, and the National Cancer Institute. Most if not all of these communications were referred to the Research Information Branch for acknowledgment or reply, and totaled approximately 1,500 over a period of nine months. So great was the volume of correspondence that an emergency operation had to be organized to deal with it and with a growing backlog of other inquiries. One RIB staff member was temporarily detailed to this work and two writers and a typist were employed on WAE appointments to augment the public inquiries group of the Information and Education Section. The temporary task force on public

inquiries was organized and directed by the Head of the Visuals, Publications, and Reference Section who also worked out a system for handling an anticipated permanently increased level of inquiries and requests under an expanded cancer research program.

As a member of the senior management staff of the Institute, the Chief of the Branch was directly involved in plans and discussions concerning possible directions that an expanded cancer research program might take. This involved sessions with the Director and his immediate staff, attendance at a meeting of top scientific consultants, and participation in a two-day staff retreat at Airlie House for review and appraisal of Institute programs. The anticipated cancer research expansion was made the keynote of the Director's opening statement for the fiscal 1972 appropriation hearings, which was drafted by the Branch Chief.

"Progress Against Cancer" TV Film

The project for production of a film for television based on the series of "Progress Against Cancer" reports, conceived by the Branch Chief early in 1968, finally came to fruition with the awarding of a contract to Wexler Film Productions of Hollywood, California in April. Work on the production, for which Danny Thomas is Executive Producer as a member of the National Advisory Cancer Council and the Council's Editorial Committee, began that month with the filming of participating NCI staff scientists and program directors, and laboratory scenes at Bethesda. The schedule set forth in the contract calls for completion and clearance of the film in time to show the finished product to the Council at its special program review meeting of October 4-5. It is anticipated that the film, entitled "Progress Against Cancer," will be on the air during the fall and winter television season. Mr. Thomas has a commitment from ABC to use it on that network, and hopes to get the cooperation of the other networks as well. The Chief of the Branch is Project Officer for the production.

Preparation of "Progress Against Cancer 1971" went forward meanwhile, with completion of a draft in the spring, and the expectation of approval by the Council at its June meeting. This report, which concentrates on the curability of cancer, will then go to press at the Government Printing Office in anticipation of delivery and release within the calendar year. Distribution of approximately 370,000 copies of "Progress Against Cancer 1970," which was mainly on the subject of chemical carcinogenesis, was completed.

Other TV Activity

The information staff gave assistance in the development of several television features for broadcast in this country and abroad. For a program on medicine sponsored by Standard Oil of Ohio, scenes were filmed in Building 41 and in the monkey colony at Litton's Bionetics Research Laboratory, an NCI contractor. MEDCOM, a private company planning a series of films on chronic diseases for possible television syndication, did filming in the viral etiology

and chemotherapy areas. A camera crew from BRT (Belgian television) visited NCI to film laboratory scenes in the carcinogen screening area for a series of one-hour programs on science, and Italian National Television brought a camera crew to Bethesda to film scenes for a documentary on smoking and health.

Magazine and News Coverage

In the magazine field, Newsweek was assisted in researching material for an article on the Cancer Chemotherapy National Service Center which appeared as the lead story in the magazine's Medicine Section in the issue of August 31. A writer for the Johannesburg, South Africa Star was briefed for a series of articles on NCI research programs, and a medical science reporter and columnist for the London Times was briefed for a story on virus-cancer research. An article on leukemia based largely on NCI staff interviews was published in the October Today's Health and subsequently in Reader's Digest. The Digest version included a special note stating that NCI had sufficient funds to care for all the leukemic children in the United States, which brought in a large volume of requests from parents who were thus misinformed. Suitable replies to hundreds of these pathetic letters were made by the Research Information Branch.

Liberal coverage was given a news release issued jointly by NCI and NIAID in July reporting the findings of NCI grantees, Drs. Howard Temin and Satoshi Mizutani of the University of Wisconsin, and Dr. David Baltimore of M.I.T., on the enzyme RNA-dependent DNA polymerase. This coverage was given by news services, news magazines, radio and television, the medical press, and the daily newspapers, including a page-one story in the New York Times. In November Dr. Robert C. Gallo of the NCI scientific staff reported evidence of a difference between normal and leukemic human cells, and a possible method of utilizing this difference in the treatment of acute leukemia patients. The news media were particularly interested in Dr. Gallo's disclosure that the RNA-dependent DNA polymerase enzyme found in the cells of leukemia patients could be blocked by a derivative of an antibiotic called rifampicin. The announcement was widely covered by the daily press, the wire services, the periodical press, and the electronic media. Two background statements on the work were issued by the Research Information Branch.

A news release issued in August on the latest results of a four-drug treatment of Hodgkin's disease developed by Drs. Paul Carbone and Vincent T. DeVita of NCI also elicited unusual interest from science writers. Requests for further information, photographs, and interviews were received from Modern Medicine, Medical World News, Medical Tribune, and the Associated Press. The Research Information Branch also cooperated with the Department of Public Information, Princeton University, in issuing an advance news announcement and assisting with news coverage of a luncheon ceremony at the Shoreham Hotel in Washington at which Dr. Robert J. Huebner of NCI was a recipient of the 1970 Rockefeller Public Service Awards.

Assistance with Scientific Meetings

The public information staff provided news coverage services for three scientific meetings held in the Washington area during the year. One was a meeting in November in the series of new drug seminars conducted by the Institute to familiarize physicians with newly available anticancer agents. It was conducted in the NIH Clinical Center, and was on the subjects of Mithramycin, to be marketed as Mithracin by Chas. Pfizer & Company, and ortho para 'DDD, to be marketed as Mitotane or "Lysodren ® " by Calbiochem. An NCI information specialist assisted in the news room operation for the International Conference on Adverse Reactions Reporting Systems in October, sponsored by the Drug Research Board of the National Research Council, National Academy of Sciences-National Academy of Engineering, and a news room operation was conducted for the 21st annual meeting of the Tissue Culture Association in June. The Branch arranged two press conferences for interviews with Dr. William Hammond, Chief of the Clinical Investigations Branch, prior to the Symposium on Statistical Aspects of Protocol Design in Puerto Rico in December. Also, assistance with news coverage of the White House Conference on Children in December was given by a member of the Research Information Branch staff.

As in past years, at the request of the American Association for Cancer Research the Branch assumed responsibility for all preparations and the conduct of news room operations for the Association's annual meeting, held in Chicago in April. This included the preparation of advance press summaries of papers selected by the Association, and arrangements for two news conferences with scientists on the program for the meeting.

Discussions were initiated with the Leukemia Society of America for joint participation in arranging news coverage services and facilities at the Vth International Symposium on Comparative Leukemia Research to be held in Padua and Venice, Italy September 13-17, 1971. Dr. Luigi Chieco-Bianchi, Vice-Chairman of the World Committee for Comparative Leukemia Research, was asked to supply information on program content and physical arrangements for the meeting, and plans were discussed with Dr. Frank J. Rauscher, NCI Scientific Director for Etiology; Dr. Ray M. Dutcher, Chairman of the World Committee; and information staff of the Leukemia Society.

Other Activities

The Research Information Branch prepared an information paper on the state of the art in cancer research and the extent of progress against the leading forms of cancer for the National Panel of Consultants on Cancer which served as an advisory group to the Senate Committee on Labor and Public Welfare to conduct studies which led to preparation of the bill introduced in the 91st Congress by Senator Yarborough for the establishment of an independent National Cancer Authority. The Branch prepared a comprehensive article for the Director on cancer prevention, detection, and treatment, which was requested for the new edition of the Encyclopaedia Britannica. Also, an article on "Opportunities for Progress Against Cancer" was prepared for the Director as requested for the Annals of the D.C. Medical Society.

In response to a request from the American Association for the Advancement of Science, the information staff compiled a list of research findings by NCI scientists, grantees, and contractors, and of NCI program developments covering the years 1945-1970.

The reports of the Sections comprising the Research Information Branch follow.

Information and Education Section

The mission of this section--to adapt information on progress in cancer research to the public--was highly influenced during the past year by the most enormous backlog in its history of unanswered inquiries from the public--most of them dealing with cutbacks in cancer research funds and many of them generated by organized campaigns of well-directed groups of citizens interested in the subject. With the assistance of other members of the Branch staff, and two writers and a typist recruited under the WAE (when actually employed) mechanism, this backlog was reduced to manageable proportions. Although the President called on Congress for greatly increased funding of cancer research in January and announced his Cancer Conquest Program in February, letters continued to be received on this important subject. A large portion of this mail was composed of controlled letters addressed to the President, the Secretary of Health, Education, and Welfare, or Members of Congress--all of which require individual and carefully drafted responses. Patient letters, and general inquiries also continued to come in at an unprecedented rate.

Other business went forward on a number of fronts--through the printed and electronic news media, published reports, and other means.

Assistance to News Media

During the year the Section was instrumental in assisting the news media--both printed and electronic--in creating greater visibility for the Institute. A total of 88 telephone inquiries by the press was answered on a variety of subjects dealing both with the scientific aspects of cancer and the fiscal or policy picture thereof. Personal conversations were held on some of these subjects with reporters and editors as wide-ranging as persons from the NIH Record to the science correspondent of the Johannesburg, South Africa Star. Others included such press stalwarts as Matt Clark, Science Editor of Newsweek, John M. Mecklin, of the Editorial Board of Fortune, and Bill Small, of Biomedical News. Dr. Frank Field, Science Editor of the National Broadcasting Company, expressed particular interest in a forthcoming press conference on a "new" skin test for cancer to be announced in April by Dr. Jack George Makari, its developer, via the press-conference route. Although this query required considerable NCI research, we were unable to be of assistance, and, thus far, no definite results arising from the test have been announced.

The Section sent out a total of 26 news releases and articles during the year. Three of the releases dealt with appointments to the National Advisory Cancer Council of Dr. Arnold L. Brown, of the Mayo Clinic; Dr. Philippe Shubik, of the Eppley Institute, University of Nebraska; Mr. James S. Gilmore, of Kalamazoo, Michigan; and Dr. Kenneth L. Krabbenhoft, Wayne State University. Another concerned a contract signed with the Stanford Research Institute concerning the identification of carcinogens in the human environment. A release announced several high-level appointments to the staff of the Institute. Awards, staff appointments, retirements, appeals for blood and certain types of patients for the NCI clinical staff were also included.

Four NCI columns appeared during the year in the NIH Feature Service, syndicated to newspapers throughout the country. In the field of electronic journalism, the Section was instrumental in the production of 13 television and 6 radio shows. Among these were "Medicine," a half-hour presentation promoted by the Standard Oil Company of Ohio and broadcast in the area covered by that company--in excess of 30 stations. This production featured the Scientific Director for Etiology, NCI, as well as the Director, NIH, and a representative of the National Library of Medicine. Another show was developed by "Medcom," a half-hour program featuring the Scientific Directors for Etiology and Chemotherapy. It will be targeted mainly at physicians in industry and other situations, and will be accompanied by a handsome biomedical brochure for each viewer.

Other air-time shows included spots or longer segments on "Discussion: NIH," Station WGMS, Washington (Radio); "NIH Reports," WRC, Washington and syndication (Television); "Panorama," WTTG, Washington.

Public Inquiries

The Section answered 4,713 public letter and telephone inquiries--the greatest number, as noted above, dealing with cutbacks in funds for cancer research. Others dealt with cancer or related problems; questions on proved or unproved methods of cancer treatment; unsolicited theories, methods, or materials for detecting or curing cancer; requests from students for cancer information or specimens for school science projects; requests from others for information on cancer, including bibliographies, or inquiries about the role of environmental or personal factors in the cause or prevention of malignant diseases. An article for Today's Health, reprinted in Reader's Digest, including an incorrect paragraph stating that NCI had sufficient funds to provide care for all U.S. leukemic children, added considerably to the volume of incoming mail.

In the area of Congressional inquiries, the Section handled 336 telephone calls or letters on a wide variety of subjects. Many of these inquiries included requests for a great variety of published materials, the bulk of which were originally issued by this Branch. They ranged from cancer-patient-problems to urgent fiscal matters. Many of the letters--as stated above--continued to urge the Federal Government to divert funds from military purposes, atomic energy, and space flights to a much more highly accelerated program of medical--and particularly cancer--research.

The Section's responsibility is to inform the public, the media, the medical community and the Congress of scientific developments in the field of cancer research. The Section is charged with obtaining and disseminating this information in the most meaningful way to its various audiences.

Throughout the year, the Section Head and Section members prepared reports, releases, press summaries and publications reporting developments of significance in cancer research by NCI's scientists, grantees, contractors, and others. Particularly close attention was given by the Section Head and Section members to areas assigned for individual coverage: chemotherapy; virus research; epidemiology and biometry; and the NCI intramural research program designated General Laboratories and Clinics. Individual members of the Section frequently served as consultants to other members of the Research Information Branch, providing liaison and information in their own assigned areas of program coverage.

In addition to these duties, several members of the staff were assigned to especially challenging projects, including a five-month assignment of one Section member to research and write a 6,675-word article on cancer for the Encyclopaedia Britannica, completed in April 1971.

To overcome day-to-day manpower shortages resulting from such projects, on several occasions writers from the Information and Education Section and writers employed by RIB with the WAE mechanism assisted in carrying on the Research and Program Reports Section's responsibilities.

Publications

Several important new publications were prepared by Research and Program Reports Section members during the year.

A research report titled Research on Malignant Diseases of the Brain was published during the year, the latest in a NCI series that includes Progress Against Leukemia, Virus-Cancer Research, and Drugs vs. Cancer.

The Cancer Story, a popular version of Dr. Michael B. Shimkin's "Science and Cancer" text, was revised extensively during the year and published in March 1971. Revisions included several new illustrations and updated research information throughout. Dr. Shimkin's own revision of the original Science and Cancer was published last year.

A new NCI pamphlet, Hodgkin's Disease, was prepared in 1970 and published in January 1971. The entirely new booklet was necessitated by rapid advances in research on causes and treatment of this form of cancer.

Revisions of site pamphlets on cancer continued as needed. Newly published during the year were Cancer of the Uterus, Cancer of the Bladder and Cancer of the Breast.

Preparation of a new site pamphlet, Cancer of the Pancreas, was undertaken as a result of increasing demands for information on this rapidly rising factor in cancer mortality in the United States.

"Progress Against Cancer" Publication

A major project of the Section continues to be the production of an illustrated publication called Progress Against Cancer, issued periodically under the auspices of the National Advisory Cancer Council.

A member of the Section's writing staff is assigned full-time to the responsibility of gathering information for and writing this report, with guidance from the Chief, Research Information Branch.

The latest report of the series, Progress Against Cancer, 1970 described progress in the field of chemical carcinogenesis. Published in December 1970, the 98-page report described research on environmental and industrial exposures to cancer risk; a major portion of text described research on tobacco carcinogenesis.

The next issue, to be subtitled "The Curability of Cancer" upon recommendation of the Council, will describe the principal means now available to control cancer -- surgery, radiotherapy, drug therapy, and supportive care. In addition, the promising new field of immunotherapy will also be surveyed.

Assistance to the News Media

Members of the Section responded to approximately 300 inquiries from members of the news media during the year, the requests rising steadily month by month as news stories on cancer research and the proposed expansion of the national cancer research effort mushroomed in the printed and electronic media.

Prior to and during hearings on the NCI budget for fiscal year 1972, various news organizations gathered data for their stories on the best method of organizing an expanded cancer research program in the U.S. Many newsmen sought budgetary information; others wanted in-depth information on existing programs as well; others merely wanted help in scheduling interviews with leading NCI scientists and administrators.

Throughout the year newsmen sought the latest information on anti-cancer drugs, and on developments in molecular biology that shed light on the causes of cancer. They asked about saccharin, moon dust and cancer-detection tests;

leukemia, breast cancer and skin cancer; the SST, BCG, and BCNU; chemotherapy in general, rifampicin derivatives in particular, and the success of some drug combinations in controlling certain forms of cancer.

After budgetary and administrative subjects, a report in November 1970 by Dr. Robert C. Gallo of NCI on a rifampicin derivative generated the second greatest number of press calls to this Section. Speaking at the Pasteur Institute in Paris, Dr. Gallo reported that n-demethyl rifampicin, a compound produced in Italy by Gruppo Lepetit Co., was able to inhibit completely, in test tube experiments, an enzyme that had been linked with the leukemias of animals and of man. Hope for a new anti-cancer drug sparked wide interest.

A major scientific advance, the discovery of an enzyme called RNA-dependent DNA polymerase, reported by NCI grantees Drs. Howard M. Temin and Satoshi Mizutani at the University of Wisconsin generated interest and inquiries throughout the year. Their discovery was described in an NCI release that resulted in coverage by Associated Press, The New York Times, The National Journal, Drug Research Reports, and The National Observer, to name a few.

Press queries on individual drugs or potential drugs, such as ICRF-159 and a platinum compound, were routine events. A round-up story on the NCI chemotherapy program appeared in Newsweek August 31,.1970.

Section members cooperated with electronic media representatives on many occasions, including preparation of two one-hour programs on cancer research by the Belgian TV station BRT; an ABC-TV network story on cancer research and its funding; taping of 10 NCI interviews by the Voice of America; production of six radio shows on cancer research in the "OF MEN AND MOLECULES" series subtitled "Cancer: Moving to Higher Ground."

Inquiries on leukemia continued throughout the year; among the media assisted were the New York Times, Baltimore News American, Medical World News and Pageant. Information was continually provided to The Candlelighters, an aroused group of parents of children with cancer, who gathered data for magazine articles and other literature they wrote, and for radio/TV interviews in which they participated.

Section members frequently assisted in press room activities, aiding newsmen in groups instead of singly. On October 22 and 23 a Section member assisted in the press room of the International Conference on Adverse Reactions Reporting Systems, held in the U.S. Department of Commerce Auditorium.

In November 1970 Section members arranged and staffed a press room at a NCI-sponsored meeting on the drugs o,p'-DDD and mithramycin, held at the Clinical Center November 5-6. Detailed fact sheets were prepared in advance on the 2 drugs, and assistance given to reporters from JAMA, Medical Tribune, Medical World News, U.S. Medicine and Drug Research Reports.

In December 1970 Section members arranged two press briefings for members of the medical press on a Symposium on Statistical Aspects of Protocol Design held in Puerto Rico December 9 and 10.

In April 1971 major assistance was given to science writers for the 12th successive year when Section members handled press relations for the annual meeting of the American Association for Cancer Research in Chicago April 8-10. In advance of the meeting, requests were sent to 30 scientists selected by the Program Committee, inviting them to submit press summaries for use by the news media. Two formal press conferences were arranged in advance, and numerous others were scheduled during the course of the 3-day meeting. Assistance of various types was provided to the 25 newsmen who attended, representing network TV, radio, wire services, medical press and daily papers.

Press Releases, Press Summaries, Reports

Section writers prepared more than a dozen news releases during the year on a variety of subjects. In July 1970 a release was issued on NCI grantee Dr. Howard Temin's momentous discovery of an enzyme associated with RNA tumor viruses that permits the transfer of genetic information from RNA to DNA. In November 1970 an announcement was made of Dr. Robert J. Huebner's extension of the "oncogene" theory to include its role as a normal growth factor in the developing embryo. In Spring 1971 a release was issued on Dr. Temin's proto-virus hypothesis described in a precedent-setting editorial in the Journal of the National Cancer Institute.

Other news releases were issued concerning a 4-drug treatment for advanced Hodgkin's disease; the transfer of cancer immunity from one animal to another; the usefulness of the drug imidazole carboxamide; a meeting held to discuss 2 new anti-cancer drugs; and an international study to identify women with a high risk of breast cancer. Releases also were prepared to announce new NCI publications and upcoming scientific meetings.

In cooperation with Princeton University's information staff, Section members helped to publicize the 1970 Rockefeller Public Service Award presented to Dr. Huebner in December 1970.

In December 1970 a press summary was prepared concerning a major talk on the future of vaccines against cancer, delivered by Dr. Frank J. Rauscher,Jr. of NCI at an International Conference on the Application of Vaccines Against Viral, Rickettsial, and Bacterial Diseases of Man. The conference was co-sponsored by the World Health Organization and the Pan American Health Organization.

Thirteen press summaries were prepared for the spring 1971 scientific meetings. These included one for use at the American Society of Clinical Oncology, Chicago; six for the American Association for Cancer Research, Chicago; four for the Federation of American Societies for Experimental Biology, Chicago; and two for the American Society for Microbiology, Minneapolis.

During the course of the year, Section writers contributed about a dozen reports to the Weekly Report to the Director, NIH. They included scientific reports concerning cancer of the prostate and drug treatment of melanoma; and program reports on awards to NCI scientists, cancer conferences, and program development news.

In September 1970 the Section Head prepared a 22-page report titled "Cancer In The United States," upon the request of a member of the Committee of Consultants for the Conquest of Cancer. During the year Section members also prepared lengthy reports for the White House, the Science Adviser to the President, and the American Association for the Advancement of Science.

In January 1971 a 24-page report on highlights of 1970 research was prepared by the Section Head for inclusion in an NIH document forwarded to the Congressional subcommittee on appropriations.

Literature Reference and Analysis

Each month more than 350 scientific journals were scanned by a Section member for reports on research findings financed by the Institute. An average of 78 references per week were noted and recorded for Branch writers and other members of the Institute staff. A monthly report was made to the American Cancer Society on scientific papers describing research supported jointly by NCI and ACS.

The same Section member completed the annual assignment of preparing the NCI portion of the NIH Scientific Directory and Annual Bibliography. This publication lists the key personnel by organizational structure and lists all papers published by NCI scientists throughout the year.

This Section member prepared bibliographies on various research topics as requested, including a bibliography appendix to the major article on cancer research for the Encyclopaedia Britannica.

Training

The value of training is recognized in the Section, and Section members are encouraged to improve their skills and effectiveness through training sessions.

The Section Head took an Advanced Course for Supervisors titled "Supervision and Group Performance" September 28 through October 2, 1970. The 40-hour course was designed by the Civil Service Commission and offered on the NIH campus by the Office of Personnel Management's Training and Development Branch.

A Section writer took an evening course entitled "Cell Biology for Science Writers" in the Fall 1970 semester of the Graduate Program at NIH.

Another member of the Section's writing staff took a course entitled "Technical Writing Workshop" November 16 through 20, 1970. The 5-day course was offered by the U.S. Civil Service Commission's Bureau of Training in Washington, D. C.

In May 1971 the Section's clerk-typist took a half-day course entitled "Telephone Techniques Reviewed" at NIH.

Publications, Visuals, and Reference Section

The Publications, Visuals, and Reference Section has responsibility for planning, organizing and executing projects for the production and distribution of printed and audiovisual materials addressed to the general public as well as to professional audiences and groups within the Government. Other activities include assisting with the planning and programming of Branch projects, planning and adjustment of the Branch budget, maintenance of a reference library, handling of review and approval of nonresearch materials, and screening and circulating journals and newspaper clippings.

Publications and Audiovisuals

Over one-half million information and education printed materials were distributed to the general public, health and medical groups, schools (elementary through professional), and private and voluntary organizations. Letters of request for information on cancer totaled 9,000.

Distribution of "Progress Against Cancer 1970," a report to the public prepared for the National Advisory Cancer Council on the status of knowledge about cancer, was made to physicians, students in professional schools in the medical and public health disciplines, Members of Congress, State and local health officials, and other selected groups.

The publication, "Chilhood Leukemia, A Pamphlet for Parents," was reprinted for the ninth time, incorporating revisions by the Chemotherapy area.

"The Cancer Story," which was reprinted with revisions based on the revised edition of "Science and Cancer," has proved to be a very popular publication in schools. Nearly one million copies have been distributed since it was released in 1965.

The Section provided assistance to various Institute areas in obtaining printing, production, and distribution of charts and publications.

Lists of cancer publications available from the Institute and from the Superintendent of Documents, GPO, were kept up to date and used to answer inquiries and with exhibits. The NCI portion of the NIH publications listing booklet was also revised as requested.

A staff member provided assistance to the NCI Volunteer Equal Employment Opportunity Group and planned and arranged for the production of "NIH-NCI EEO Action."

Background material for the film, "Progress Against Cancer," was prepared for a meeting of prospective bidders. The Section head participated in the meeting. After award of the contract the Section head assisted the Branch Chief with appointments and provided visual and other materials as required by the producer.

Reproductions of each of the four program areas presented in the new exhibit, "Research and Related Programs of the National Cancer Institute," were completed for use by the areas. This exhibit and others were shown upon request. A permanent display board was designed and purchased for the 11th floor hall and several displays were prepared.

Existing films, slides, and photographs were supplied upon request. Photographs, charts, slides and other materials were prepared on a continuing basis. Assistance was provided to the media in obtaining or making photographs.

Reference Service

At the request of the Executive Officer, arrangements were made to reproduce NIH "Current Clips," augmented in the Section by clippings of special NCI interest from newspapers screened in the Branch. The "Clips" are distributed to senior NCI staff members.

Medical news magazines and other printed materials and periodicals received in the Branch were screened for articles on cancer and pertinent ones circulated to the Branch staff. These materials and other reference items are retained in the Section reference library for use by Institute staff.

Review and Approval of Nonresearch Materials

Approximately 100 nonresearch items prepared by the Institute staff for publication or other official use were received and reviewed in the Section, forwarded to the Branch Chief for his approval, and, thence, to other channels required by NIH policy and procedure directives. Monthly reports of materials cleared and in clearance were prepared and submitted to the Institute Board of Editors to be appended to its reports of research materials in clearance.

Other Activities

The Section head prepared a message for President Nixon's signature to be sent to the W. Alton Jones Cell Science Center at the time of dedication ceremonies in June.

Staff members attended information personnel seminars offered by NIH throughout the year.

In February, the Branch Chief directed the PVR Section head and a staff member to assess the mounting backlog of nonpatient public inquiry letters. Nearly 2,000 letters dating back to December 1969 were read and categorized as to subject. Standardized paragraphs were prepared to answer effectively approximately 1,500 of these letters. There were 100 requests for materials which did not require an answer other than the standard enclosure form. Nearly 400 inquiries required special replies.

To facilitate the typing of letters, a special typewriter which operates from a film negative was obtained as a demonstrator. It was found to be an extremely useful aid in typing of patient letters and others in which standardized paragraphs are employed in responses. We have recommended that a machine of this type be obtained for OD use.

To assist with the writing of the backlog of letters, two WAE employees were added to the staff and assigned to PVR. A WAE typist was also employed.

By March 15, all public inquiry letters were being answered on a current basis.

To prevent a recurrence of a build-up of letters in the Branch, a form was designed for use on a trial basis to aid in maintaining records of letter receipts, assignments, and completion. Code letter and paragraph books were prepared for use by letter writers and typists.

NATIONAL CANCER INSTITUTE
CHEMOTHERAPY

Annual Report of the Scientific Director
July 1, 1970 - June 30, 1971

Introduction

It is appropriate at this time, when the Conquest of Cancer Program (CCP) is under intense discussion, to summarize the present status of the drug control of cancer, the prospects for additional successes in the near future, and the potential impact of the CCP on the search for drugs.

Present Status of Chemotherapy

Some would summarize chemotherapy of cancer in a statement such as this: "a lot of money has been spent, and no cure for cancer has been found." This is a reckless statement that gives rise to a number of false assumptions. In the first place, it leads to the implication that there should be a single drug that will cure the 100 or more types of clinical cancer. No responsible investigator or physician working in cancer research would consider this a reasonable objective any more than they would regard as possible a single drug that would cure all infections -- malaria, syphilis, measles, staphylococcus infections, etc. Secondly, this kind of statement might lead some to assume that since one drug can't cure all cancer patients, that therefore, there are no curative drugs for any single type of cancer. This latter implication is manifestly false, since there are a number of drugs that will cure specific kinds of cancer.

By cure I mean that the life expectancy of the treated cancer patient is the same as "normal" life expectancy, specifically, the same as that of a matched cohort in the general population. Evidence exists to show cures from drugs alone by this definition in choriocarcinoma, Burkitt's lymphoma, acute lymphocytic leukemia and testicular cancer. Recent studies have shown that cures of the same sort are very likely occurring in advanced Hodgkin's disease, lymphosarcoma and mycosis fungoides, but another year or two of observation is required for certainty. Finally, there are a series of childhood tumors (Wilms', Ewing's and rhabdomyosarcoma) where cure rates are low after treatment by x-irradiation or surgery alone, or in combination, and where the addition of drugs has resulted in a high percentage of cures. The drugs used in the above curative situations are:

Methotrexate
Mercaptopurine
Nitrogen Mustard
Actinomycin D
Matulane
Cyclophosphamide
Vincristine
Prednisone
Mithramycin

In all, there are 40 drugs that are active against cancer, representing - when analogues are subtracted - 24 different chemical structures. Many of these agents are also active in the curable tumors and many of them show substantial activity - though not in the curative range - in the more common tumors such as carcinomas of the colon and breast. One immediately asks why some types of tumor are curable by drugs and others not. A great deal of work in the last few years has shown that sensitivity to drugs is related to growth rate of the tumor and more fundamentally to the percentage of cells of the tumor going through DNA synthesis at any one time (the growth fraction). This is a gross over simplification and there are many caveats, but the general principle of a high degree of drug sensitivity of rapidly growing tumors is true and these interrelationships have provided a rational base for cancer chemotherapy. Since previous annual reports have reviewed the rational base, I will omit further discussion.

There has arisen then, in the last few years, a modern chemotherapy that has resulted in a potential for cure of one large group of cancers (about 15% of clinical cancer) and given rise to a theoretical framework that promises better results in the 85%. Let me define this modern chemotherapy. It starts with the assumption that when clinical disease is apparent, a large number of tumor cells, something of the order of a trillion (1×10^{12}), is present in the patient. All of these cells must be killed in order to cure. Drugs will kill a constant percentage of the dividing tumor cells during each administration. They will also kill normal cells that are dividing. However, normal cells have a greater capacity to recover from drug insult than rapidly dividing tumor cells, so it is possible to engineer intermittent courses of drugs to kill far more tumor cells than normal cells. It has also been shown that combinations of drugs can be selected that will be additive in their killing power of tumor cells, but not additive against normal cells. In fact, in most modern chemotherapy, combinations are essential; the only exception being the exquisitely drug sensitive choriocarcinoma and Burkitt's lymphoma. A knowledge of the pharmacology and toxicology of the several drugs permits drug scheduling that will exploit their selective killing capacity for the tumor cells. Finally, the balance of selective toxicity can be further tipped in favor of the patient's normal cells by various supportive measures that make up for the deficit caused by the drugs' effects on normal cells. For example, if the drug destroys platelets, these are replaced.

Modern chemotherapy, does not merely consist of routine ordering of a drug, but is made up of rather complex and sophisticated total management requiring a good deal of information on cell population kinetics of normal and tumor cells, on toxicology and pharmacology, and on the physiology of cell replacement. It clearly demands a highly trained team whose members are prepared to make all the measurements required to give drugs and other therapy so that a trillion tumor cells can be killed without the death or serious damage to the patient.

Prospects for Additional Successes

Prospects for widening the chemical control of cancer seems bright, both for immediate and longer term (one to five years) objectives. In the immediate category are application of modern chemotherapy to all patients with curable

tumors; the broadening of combination studies; and the pursuit of different types of agents coming from new screens. In the delayed category are solution of several specific obstacles to cure and better prediction systems.

Application of Modern Chemotherapy

While approximately 15% of clinical cancers could now be cured by the use of drugs or the addition of drugs to other therapy, only a small fraction (?20%) of those who could benefit actually have an opportunity to receive modern chemotherapy; and here the NCI faces a problem that cannot be solved through existing mechanisms. On the one hand, the NCI, through its research cadre and consultants, knows how to create the special circumstances needed for modern chemotherapy; and must, therefore, take the initiative in further application. On the other, it seems untraditional for a research management group in government to take the lead in what could be construed as delivery of medical care. The need really isn't for delivery of care so much for as an instrument for a sudden expansion of hospitals with a favorable climate for the requisite trained teams, plus a rapid nationwide educational program on modern chemotherapy. Provided that the necessary elements can be brought together successfully, opportunity is at hand to make curative chemotherapy widely available. This can be done by the expansion and addition of cancer centers where the needed interface can be established between the trained team and the appropriate segments of the health delivery team.

Use of Combinations of Drugs

The power of modern chemotherapy is best seen in the use of 4 drug combinations in acute lymphocytic leukemia and advanced Hodgkin's disease. Even in these classic situations, x-irradiation (Pinkel's use of prophylactic irradiation for the prevention of CNS leukemia) and surgery (early splenectomy in Hodgkin's) are recent welcome additions. The philosophy is of course to rid the body of tumor cells by optional combinations of the best means at hand. These principles have yet to be applied to the great majority of tumors, and while there are obstacles, the research knowledge for a number of other tumors is sufficiently within grasp that application is a problem of logistics rather than fundamental new knowledge. Recent combinations of classic drugs have shown improved results in breast cancer and the oat cell variety of lung cancer. However, more studies are needed of drug combinations, both drugs alone, and in conjunction with irradiation and surgery. With a number of new drugs shown to be clinically active, there are additional opportunities for more effective combinations. Finally, the use of drug combinations after surgical excision of tumors should be tried in those patients whose operative findings strongly suggest a poor prognosis.

Pursuit of Drugs Coming from New Screens

The recognition that growth rate and growth fraction of tumors were determinants of drug susceptibility led to the study of a number of slow-growing (small growth fraction) tumors in animals. Several of these have been developed to the point that make them suitable as additional animal screens. While it will be another year before these new screens will be in full operation, new drugs of high effectiveness have been uncovered. These will be studied in the

common, slow-growing tumors in the clinic this year. However, the longer term strategy will be to screen many drugs in these new screens with the conviction that the biology of these animal tumors is closely similar to that of the common tumors in man, and the hope that the screens will identify chemicals uniquely capable of killing slow-growing tumors.

Removal of the Classic Obstacles to Curative Chemotherapy

These obstacles were detailed in last year's annual report and consist of lack of a quantitative chemical assay for residual tumor cells in the patient, after therapy; methods for assay of the cell population kinetics of tumor and normal cells; better ways to treat tumor cells pharmacologically protected within the central nervous system (CNS) and regimens with greater selective toxicity for tumor cells. Progress has been made in the assay problem and studies are underway using immunologic (Gold's fetal antigen) and biochemical markers (Russell's polyamines and Gallo's methylated bases of tRNA). The CNS hideouts are being attacked by the development of highly lipid soluble drugs with easy access across the blood brain barrier. The best of these in animals is methyl-CCNU, now ready for clinical trial. Finally, a major effort has just been completed concerning the total problem of the selective toxicity of arabinosyl cytosine (Ara-C). This analysis involved the cell population kinetics of leukemic cells, normal bone marrow cells, pharmacologic disposition and therapeutic and toxicologic effects in man and four animal species for a wide variety of dose schedules. The insights derived from this study of how selective toxicity is increased have been applied successfully to acute myelocytic leukemia and have laid the ground work for a general theory of host - tumor cell - drug interactions in a way that facilitates inter-species conversion of data obtained in a single species. While the work is by no means complete, the progress toward a general theory has been considerable.

Better Prediction Systems

Better drugs for controlling cancer must come from in vivo or in vitro prediction systems that signal the activity of a new agent against tumor cells or some biochemical event essential for tumor growth. The NCI has learned an enormous amount about the practice and theory of screening during the last 15 years. There now exist biologically sound mouse screens for both major categories of cancer -- the large and small growth fraction tumors. In addition, during the past year, there have emerged a number of other opportunities for different approaches to prediction of drug activity and research in these areas is intense. In vivo there has been developed a way of using the spontaneous AKR leukemia to assay drug effects. This spontaneous tumor has been most resistant to chemotherapy and provides the best model yet to simulate clinical acute leukemia. A somewhat analogous model has been developed using Moloney virus in the balb/c mouse. Both of these are ideal for studying effects of immunotherapy and anti-viral therapy (such as rifampacin derivatives). Also a primate model for CNS leukemia has been developed in the owl monkey using Herpes saimiri. In addition, there are many developments in in vitro screening - biochemical screens - such as the RNA dependent DNA polymerase; cell kinetic screens - such as cultures synchronized in G_1; organ culture screens - such as breast cancer tissue; and mathematical models. None

of these has a known clinical correlate, but drugs coming from such research efforts, if different from the drugs found by in vivo screening, will be tried in man in order to scrutinize such correlation.

Impact of CCP on the Chemotherapy Program

As the several elements on the Conquest of Cancer Program begin to take shape, it is not inappropriate to wonder whether the CCP will hasten the chemical control of cancer. Certainly at first glance, the extra dollars and positions bolster the program. The difficulties of lessened support for chemotherapy grants are well known. Just as critical has been the diminished support for the NCI chemotherapy program. This began in FY 1966 and by FY 1971 the fewer dollars plus inflation had cut the program almost in half. Extensive program reductions were carried out and in some instances these have had substantial effects. For example, both the number of compounds coming in to the screen, and the kinds of tumors in the screen were sharply reduced. Since there is a 2-7 year lag between initial observation and clinical trial, the reduction in numbers of drugs for the clinic is only now beginning to be felt. However, because of the lag, we are now in for two or three lean years as far as new candidates for clinical trial. In spite of all the opportunities presented for better prediction systems, a sudden doubling of screening tests and in the number of compounds coming into the screens would have no effect on the clinic until 2-5 years from today. The staff has underway an intensive study of how the new dollars and positions could be used to reduce the lag time so that clinical candidate drugs will continue to flow. Certainly in this and other similar situations, the increased resources will speed up the search for new drugs.

The impact of the CCP will, however, be determined not so much by the totals in dollars and staff, as by the way in which these resources are used, and the only way in which they can be used with greater effect is within a highly unified program. Within the NCI chemotherapy program there is some evidence that this unity is a reality in terms of the classic criteria - definition of specific objectives, a logical plan for drug development, a system of operations based on this logic, sharp decision making and placing of priorities, resource flow in accordance with priorities, opportunity for fundamental research, close integration of in house research and the drug development program, and good coordination with some of the essential elements in the clinical trials grant program.

When we examine the total research capacity of the country in cancer chemotherapy in light of these criteria, it is apparent that such a unified program does not now exist; and, of course, probably a majority of scientists hold that there should be no unity of effort. There is clearly a ready source of conflict between the mission oriented, disease oriented direct program, and the discipline oriented study sections and university departments. If this philosophical gulf is not bridged, the increased resources of the CCP will in part be wasted. There is a need for a serious and gracious effort on the part of the scientific, clinical, and administrative communities, both within and without the NCI, to define the nature of the effort and make it possible for all to work in that effort. It will require a willingness to work together to define the key objectives (including the objectives of

complete independence for some segments) and to use every means to reach these objectives without waste of time or effort. There is an urgency to find such common ground. The degree to which the CCP nurtures a unified and dynamic effort will determine the ability of our country to seize upon the elegant opportunities for drug control of cancer.

TABLE I
ANALYSIS OF CONTRACT ACTIVITIES IN
THE CHEMOTHERAPY PROGRAM

	TYPE OF ACTIVITY	NO. OF CONTRACTS	ANNUAL LEVEL[1]	(PERCENT)
	TOTAL	129[2]	$20,887,000	
I.	DRUG PROCUREMENT		3,457,000	(16.5)
	A. Preparative Laboratories			
	1. Clinical	6	$ 779,000	(3.7)
	2. Non-Clinical	5	382,000[3]	(1.8)
	B. New Synthesis	7	1,428,000	(6.9)
	C. Dosage Formulation	4	338,000[3]	(1.6)
	D. Quality Control	4	530,000	(2.5)
II.	NATURAL PRODUCTS DEVELOPMENT		1,563,000	(7.5)
	A. Antibiotic Fermentation & Isolation	2	378,000	(1.8)
	B. Plant & Other Natural Product Collection & Isolation	8	555,000	(2.7)
	C. Related Assay Testing	9	626,000	(3.0)
	D. Production for Pharmacology & Clinic	1	4,000	--
III.	DRUG EVALUATION		3,966,000	(19.0)
	A. Primary In Vivo Screening	7	1,133,000	(5.4)
	B. In Vitro Screening	6	225,000	(1.1)
	C. Detailed Evaluation of New & Old Drugs	7	1,025,000	(4.9)
	D. Toxicology and Pharmacology	10	1,583,000	(7.6)
IV.	CLINICAL TRIALS		3,766,000	(18.0)
	A. Patient Supportive Care	6	755,000	(3.6)
	B. Clinical Experimental Therapeutics	12	2,695,000[3]	(12.9)
	C. Miscellaneous	5	316,000	(1.5)
V.	ANIMAL PROCUREMENT & SUPPORTING SERVICES		3,539,000	(17.0)
	A. Animal Production	36	2,442,000	(11.7)
	B. Disease Control	4	267,000	(1.3)
	C. Trust Stocks & Genetic Control	9	767,000	(3.7)
	D. Tumor & Cell Culture Banks	2	63,000	(0.3)
VI.	RESEARCH SERVICES		826,000	(4.0)
	A. Data Processing	7	737,000[3]	(3.6)
	B. Literature, Publications and Related Activities	3	69,000[3]	(0.3)
	C. Miscellaneous	1	20,000	(0.1)
VII.	RESEARCH AND DEVELOPMENT		3,770,000	(18.0)
	A. New Screening Methods	9	1,546,000	(7.4)
	B. Biochemical Mechanisms of Action and Resistance	5	537,000	(2.6)
	C. Pharmacology & Physiological Disposition	9	1,521,000	(7.3)
	D. Immunity & Immune Response	1	135,000	(0.6)
	E. Miscellaneous	1	31,000	(0.1)

[1] Funds obligated during Fiscal Year 1971. Not included are funds utilized for direct purchase of clinical drugs (approximately $965,000) and about twenty-five new contracts not negotiated as of this report ($3,784,000).

[2] Since many contracts involve more than one activity, the totals of I -VII do not correspond with the total number of contracts.

[3] Not included in this amount is the cost of one or more on-going contracts in which no Fiscal Year 1971 funds were obligated. See Table II for individual contracts involved.

TABLE II
ANALYSIS OF CONTRACTS
BY ACTIVITY

ACTIVITY / CONTRACT		DOLLAR LEVEL[1]
I. DRUG PROCUREMENT		$3,457,000
A. Preparative Laboratories		
1. Clinical		$ 779,000
Aerojet General Corp. (68-1300)	$ 140,000	
Collaborative Research (66-506)	57,000	
Merck and Company (62-479)	230,000	
Monsanto Research Corp. (65-1044)	120,000	
Monsanto Research Corp. (67-658)	147,000	
Starks Associates (62-484)	85,000	
2. Non-Clinical		382,000
Merck and Company (62-479)	219,000	
Monsanto Research Corp. (65-1044)	9,000	
Stanford Research Inst. (69-37)	(60,000)[2]	
Starks Associates (62-484)	84,000	
Starks Associates (68-1275)	70,000	
B. New Synthesis		1,428,000
Georgia Inst. of Tech. (68-1288)	30,000	
Michigan, University of (69-2004)	39,000	
Microbiological Assoc. (68-1283)	272,000	
Midwest Research Inst. (65-94)	275,000	
Southern Research Inst. (71-2021)	426,000	
Stanford Research Inst. (71-2070)	281,000	
Utah, University of (65-1041)	105,000	
C. Dosage Formulation		338,000
Ben Venue Labs., Inc. (66-909)	264,000	
Elder, Paul B., Company (69-2110)	5,000	
Michigan, University of (68-1284)	69,000	
National Drug Company (70-2227)	(137,000)[2]	
D. Quality Control, etc.		530,000
Microbiological Assoc. (68-1283)	174,000	
Midwest Research Inst. (69-2113)	124,000	
Pittsburgh, Univ. of (67-1186)	9,000	
Stanford Research Inst. (71-2070)	223,000	

TABLE II (Continued)

ACTIVITY / CONTRACT		DOLLAR LEVEL[1/]	
II. NATURAL PRODUCTS DEVELOPMENT			$1,563,000
A. Antibiotic Fermentation & Isolation		$ 378,000	
Bristol Laboratories (69-35)	$ 259,000		
Upjohn Company (68-1023)	119,000		
B. Plant & Other Natural Product Collection & Isolation		555,000	
Agriculture, Dept. of (64-1)	167,000		
Arizona, Univ. of (67-1484)	99,000		
Bristol Laboratories (69-35)	3,000		
Costa Rica, Univ. of (64-31)	11,000		
Hawaii, Univ. of (67-748)	20,000		
Research Triangle Inst. (69-2019)	120,000		
Virginia, University of (71-2099)	93,000		
WARF Institute, Inc. (67-747)	42,000		
C. Related Assay Testing		626,000	
Bristol Laboratories (69-35)	235,000		
IIT Research Inst. (68-1021)	54,000		
Little, Arthur D., Inc. (65-61)	50,000		
Michigan Dept. of Health (65-7)	35,000		
Research Triangle Inst. (69-2019)	15,000		
TRW, Inc. (63-622)	99,000		
Upjohn Company (68-1023)	25,000		
WARF Institute, Inc. (66-494)	98,000		
Wisconsin, University of (67-1126)	15,000		
D. Production for Pharmacology and Clinic		4,000	
Bristol Laboratories (69-35)	4,000		
III. DRUG EVALUATION			$3,966,000
A. Primary In Vivo Screening		$1,133,000	
Bristol Laboratories (69-35)	49,000		
IIT Research Inst. (68-1021)	247,000		
Little, Arthur D., Inc. (65-61)	200,000		
Microbiological Assoc. (68-1283)	60,000		
Southern Research Inst. (71-2098)	200,000		
TRW, Inc. (63-622)	223,000		
WARF Institute, Inc. (66-494)	154,000		

TABLE II (Continued)

ACTIVITY / CONTRACT	DOLLAR LEVEL[1/]	
III. DRUG EVALUATION (Continued)		
B. In Vitro Screening		$225,000
Bristol Laboratories (69-35)	17,000	
Little, Arthur D., Inc. (65-61)	30,000	
Miami, University of (70-2225)	59,000	
Southern Research Inst. (71-2098)	34,000	
Upjohn Company (68-1023)	73,000	
Yale Univ. School Med. (67-1193)	12,000	
C. Detailed Evaluation of New & Old Drugs		1,025,000
Bristol Laboratories (69-35)	8,000	
IIT Research Inst. (68-1021)	23,000	
Little, Arthur D., Inc. (65-61)	181,000	
Microbiological Assoc. (68-1283)	335,000	
Southern Research Inst. (71-2098)	373,000	
TRW, Inc. (63-622)	59,000	
WARF Institute, Inc. (66-494)	46,000	
D. Toxicology and Pharmacology		1,583,000
IIT Research Inst. (67-1141)	149,000	
Little, Arthur D., Inc. (65-61)	251,000	
Mason Research Inst. (70-2055)	299,000	
Medical College of Va. (69-2266)	6,000	
Microbiological Assoc. (68-1283)	157,000	
South Shore Lab., Inc. (64-932)	180,000	
Southern Research Inst. (70-2083)	314,000	
Texas, University of (67-1143)	101,000	
TRW, Inc. (69-2067)	36,000	
Yale U. School Med. (70-2089)	90,000	
IV. CLINICAL TRIALS		$3,766,000
A. Patient Supportive Care		755,000
American Natl. Red Cross (70-2005)	123,000	
California, Univ. of (70-2092)	87,000	
Georgetown U. Sch. Med. (71-2123)	77,000	
Litton Industries, Inc. (68-1287)	21,000	
Microbiological Assoc. (69-2177)	51,000	
TRW, Inc. (68-1286)	396,000	

TABLE II (Continued)

ACTIVITY / CONTRACT		DOLLAR LEVEL[1/]
IV. CLINICAL TRIALS (Continued)		
B. Clinical Experimental Therapeutics		$2,695,000
Bowman Gray Sch. Med. (68-950)	9,000	
California, Univ. of (70-2014)	20,000	
Duke University (70-2030)	12,000	
Indiana Univ. Fdn. (70-2028)	17,000	
Kentucky, Univ. of (67-1173)	5,000	
Makerere University (67-1343)	184,000	
Mayo Foundation (70-2066)	(126,000)[2/]	
New York University (67-1174)	10,000	
Ohio State Univ. (67-1175)	10,000	
Public Health Serv. (64-3)	1,350,000	
Veterans Adm. (64-4)	1,029,000	
Walter Reed Gen. Hosp. (64-5)	49,000	
C. Miscellaneous		316,000
Abcor, Inc. (70-2239)	77,000	
Amer. Cancer Society (71-743)	15,000	
Assoc. Biomedic Sys.,Inc.(70-2271)	94,000	
Bionetics Res. Lab. (71-2057)	50,000	
Natl. Bureau of Stds.(69-49)	80,000	
V. ANIMAL PROCUREMENT AND SUPPORTING SERVICES		$3,539,000
A. Animal Production		2,442,000
Antec Corporation (71-380)	13,000	
ARS/Sprague-Dawley (71-650)	85,000	
ARS/Sprague-Dawley (70-2044)	66,000	
ARS/Sprague-Dawley (71-2007)	120,000	
Battelle Memorial Inst.(69-2251)	120,000	
Bellaire Acres (71-649)	84,000	
Bellaire Acres (71-652)	44,000	
Chas. River Breed. Labs. (66-87)	50,000	
Chas. River Breed. Labs. (71-443)	45,000	
Chas. River Breed. Labs. (71-668)	44,000	
Chas. River Breed. Labs.(70-2043)	63,000	
Chas. River Breed. Labs.(71-644)	44,000	
Flow Res. Animals, Inc.(71-655)	78,000	
Flow Res. Animals, Inc.(71-656)	43,000	
Harlan Industries, Inc.(71-646)	43,000	

TABLE II (Continued)

ACTIVITY CONTRACT		DOLLAR LEVEL[1/]
V. ANIMAL PROCUREMENT AND SUPPORTING SERVICES (Continued)		
A. Animal Production (Continued)		
Horton Labs., Inc. (71-684)	44,000	
Horton Labs., Inc. (71-709)	44,000	
Jackson Laboratory (71-80)	109,000	
Kansas, University of (71-2010)	31,000	
Lab. Res. Enterprises, Inc. (71-379)	14,000	
Laboratory Supply Co. (71-641)	83,000	
Laboratory Supply Co. (71-696)	8,000	
Laboratory Supply Co. (70-2041)	90,000	
Marshall Res. Animals (71-378)	13,000	
Microbiological Assoc. (68-1283)	338,000	
Rawley Farms (71-654)	80,000	
Rawley Farms (71-669)	40,000	
Simonsen Labs., Inc. (65-55 & 71-2101)	32,000	
Simonsen Labs., Inc. (71-639)	94,000	
Simonsen Labs., Inc. (71-657)	48,000	
Southern Animal Farms (71-640)	81,000	
Southern Animal Farms (71-645)	44,000	
Taconic Farms, Inc. (71-651)	67,000	
Texas Inbred Mice Co. (68-88)	133,000	
Texas Inbred Mice Co. (71-658)	90,000	
Tulane University (65-593)	17,000	
B. Disease Control		267,000
Battelle Memorial Inst. (70-2008)	43,000	
IIT Research Inst. (68-1021)	109,000	
Natl. Acad. Sci. (64-44) (T.O.#6)	15,000	
N.J. College Med. & Dent. (66-52)	100,000	
C. Trust Stocks and Genetic Control		767,000
ARS/Sprague-Dawley (71-2007)	242,000	
Battelle Memorial Inst. (69-2251)	40,000	
Chas. River Breed. Labs. (66-87)	76,000	
Health Research, Inc. (70-2035)	72,000	
Kansas, University of (71-2010)	31,000	
Laboratory Supply Co. (70-2041)	30,000	
Simonsen Labs., Inc. (65-55 & 71-2101)	99,000	
Texas Inbred Mice Co. (68-88)	109,000	
Tulane University (65-593)	68,000	

TABLE II (Continued)

ACTIVITY / CONTRACT		DOLLAR LEVEL[1]
V. ANIMAL PROCUREMENT AND SUPPORTING SERVICES (Continued)		
D. Tumor and Cell Culture Banks		$ 63,000
Little, Arthur D., Inc. (65-61)	38,000	
Microbiological Assoc. (68-1283)	25,000	
VI. RESEARCH SERVICES		$ 826,000
A. Data Processing		737,000
Chemical Abstracts Serv. (65-1021)	119,000	
Control Data Corp. (67-1356)	(35,000)[2]	
Info. & Commun. Applic. (69-2236)	18,000	
Mason Research Inst. (70-2055)	100,000	
Southern Res. Inst. (71-2098)	25,000	
Thompson, J.I., Co. (69-2158)	22,000	
Vitro Laboratories (69-58)	453,000	
B. Literature, Publications and Related Activities		69,000
Geo. Washington Univ. (69-2116)	(3,000)[2]	
Scientific Lit. Corp. (69-2114)	(111,000)[2]	
Scientific Lit. Corp. (70-2267)	69,000	
C. Miscellaneous		20,000
Natl. Acad. Sci. (64-44) (T.O. #16)	20,000	
VII. RESEARCH AND DEVELOPMENT		$3,770,000
A. New Screening Methods		1,546,000
Atomic Energy Comm. (71-56)	17,000	
IIT Research Inst. (68-1021)	40,000	
Little, Arthur D., Inc. (65-61)	290,000	
Microbiological Assoc. (68-1283)	224,000	
Southern Research Inst. (71-2098)	600,000	
TRW, Inc. (63-622)	4,000	
TRW, Inc. (66-84)	316,000	
Upjohn Company (68-1023)	50,000	
WARF Institute, Inc. (66-494)	5,000	
B. Biochemical Mechanisms of Action and Resistance		537,000
Cornell Univ. Med. College (69-60)	80,000	
Delaware, University of (69-2115)	19,000	
Southern Research Inst. (71-2098)	55,000	
Southern Research Inst. (66-29)	271,000	
Upjohn Company (68-1023)	112,000	

TABLE II (Continued)

ACTIVITY CONTRACT		DOLLAR LEVEL[1/]
VII. RESEARCH AND DEVELOPMENT (Continued)		
C. Pharmacology and Physiological Disposition		$1,521,000
Jefferson Med. College (70-2136)	40,000	
Little, Arthur D., Inc. (65-61)	585,000	
Microbiological Assoc. (68-1283)	224,000	
Rochester, University of (69-39)	80,000	
Southern Research Inst. (65-654)	170,000	
Southern Research Inst. (71-2098)	100,000	
Texas, University of (66-1156)	198,000	
Upjohn Company (68-1023)	121,000	
Yale Univ. School Med. (67-1193)	3,000	
D. Immunity and Immune Response		135,000
Microbiological Assoc. (68-1283)	135,000	
E. Miscellaneous		31,000
Pomona College (68-2112)	31,000	

1/ Funds obligated during Fiscal Year 1971. Not included are funds utilized for direct purchase of clinical drugs (approximately $965,000) and about twenty-five new contracts not negotiated as of this report ($3,784,000).

2/ Not included in dollar totals since no Fiscal Year 1971 funds were obligated; included here for reference since it is an on-going contract.

TABLE III
ANALYSIS OF ACTIVITIES
BY CONTRACT

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Abcor, Inc. (70-2239)			
IVC	Clinical Trials (Miscellaneous)		$ 77,000
Aerojet-General Corporation (68-1300)			
IA1	Clinical Preparative Laboratories		140,000
Agriculture, Department of (Transfer - 64-1)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		167,000
American Cancer Society (71-743)			
IVC	Clinical Trials (Miscellaneous)		15,000
American National Red Cross (70-2005)			
IVA	Patient Supportive Care		123,000
Antec Corporation (71-380)			
VA	Animal Production		13,000
Arizona, University of (67-1484)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		99,000
ARS/Sprague-Dawley (71-650)			
VA	Animal Production		85,000
ARS/Sprague-Dawley (70-2044)			
VA	Animal Production		66,000
ARS/Sprague-Dawley (71-2007)			362,000
VA	Animal Production	120,000	
VC	Trust Stocks & Genetic Control	242,000	
Associated Biomedic Systems, Inc. (70-2271)			
IVC	Clinical Trials (Miscellaneous)		94,000
Atomic Energy Commission (71-56)			
VIIA	Screening R&D		17,000
Battelle Memorial Institute (69-2251)			160,000
VA	Animal Production	120,000	
VC	Trust Stocks & Genetic Control	40,000	
Battelle Memorial Institute (70-2008)			
VB	Animal Disease Control		43,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Bellaire Acres (71-649)			
VA	Animal Production		$ 84,000
Bellaire Acres (71-652)			
VA	Animal Production		44,000
Ben Venue Laboratories, Inc. (66-909)			
IC	Dosage Formulation		264,000
Bionetics Research Laboratory (71-2057)			
IVC	Clinical Trials (Miscellaneous)		50,000
Bowman Gray School of Medicine (68-950)			
IVB	Clinical Experimental Therapeutics		9,000
Bristol Laboratories (69-35)			575,000
IIA	Antibiotic Fermentation & Isolation	259,000	
IIB	Plant & Other Natural Prod. Coll. & Iso.	3,000	
IIC	Natural Product Assay	235,000	
IID	Production for Pharmacology & Clinic	4,000	
IIIA	Primary In Vivo Screening	49,000	
IIIB	In Vitro Screening	17,000	
IIIC	Detailed Evaluation of New & Old Drugs	8,000	
California, University of (70-2014)			
IVB	Clinical Experimental Therapeutics		20,000
California, University of (70-2092)			
IVA	Patient Supportive Care		87,000
Charles River Breeding Laboratories, Inc. (66-87)			126,000
VA	Animal Production	50,000	
VC	Trust Stocks & Genetic Control	76,000	
Charles River Breeding Laboratories, Inc. (71-443)			
VA	Animal Production		45,000
Charles River Breeding Laboratories, Inc. (71-668)			
VA	Animal Production		44,000
Charles River Breeding Laboratories, Inc. (70-2043)			
VA	Animal Production		63,000
Charles River Breeding Laboratories, Inc. (71-644)			
VA	Animal Production		44,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS	DOLLAR LEVEL
Chemical Abstracts Service (65-1021)		
VIA	Data Processing	$ 119,000
Collaborative Research, Inc. (66-506)		
IA1	Clinical Preparative Laboratories	57,000
Control Data Corporation (67-1356)		
VIA	Data Processing	(35,000)[1/]
Cornell University Medical College (69-60)		
VIIB	Biochemical Mechanisms	80,000
Costa Rica, University of (64-31)		
IIB	Plant & Other Natural Prod. Coll. & Isolation	11,000
Delaware, University of (69-2115)		
VIIB	Biochemical Mechanisms	19,000
Duke University (70-2030)		
IVB	Clinical Experimental Therapeutics	12,000
Elder, Paul B., Company (69-2110)		
IC	Dosage Formulation	5,000
Flow Research Animals, Inc. (71-655)		
VA	Animal Production	78,000
Flow Research Animals, Inc. (71-656)		
VA	Animal Production	43,000
Georgetown University School of Medicine (71-2123)		
IVA	Patient Supportive Care	77,000
George Washington University (69-2116)		
VIB	Literature, Publications & Related Activities	(3,000)[1/]
Georgia Institute of Technology (68-1288)		
IB	New Synthesis	30,000
Harlan Industries, Inc. (71-646)		
VA	Animal Production	43,000
Hawaii, University of (67-748)		
IIB	Plant & Other Natural Prod. Coll. & Isolation	20,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Health Research, Inc. (70-2035)			
VC	Trust Stocks & Genetic Control		$ 72,000
Horton Laboratories, Inc. (71-684)			
VA	Animal Production		44,000
Horton Laboratories, Inc. (71-709)			
VA	Animal Production		44,000
IIT Research Institute (67-1141)			
IIID	Toxicology & Pharmacology		149,000
IIT Research Institute (68-1021)			473,000
IIC	Natural Product Assay	54,000	
IIIA	Primary In Vivo Screening	247,000	
IIIC	Detailed Evaluation of New & Old Drugs	23,000	
VB	Animal Disease Control	109,000	
VIIA	Screening R&D	40,000	
Indiana University Foundation (70-2028)			
IVB	Clinical Experimental Therapeutics		17,000
Information and Communications Applications, Inc. (69-2236)			
VIA	Data Processing		18,000
Jackson Laboratory (71-80)			
VA	Animal Procurement		109,000
Jefferson Medical College (70-2136)			
VIIC	Pharmacology & Physiological Disposition		40,000
Kansas, University of (71-2010)			62,000
VA	Animal Production	31,000	
VC	Trust Stocks & Genetic Control	31,000	
Kentucky, University of (67-1173)			
IVB	Clinical Experimental Therapeutics		5,000
Laboratory Research Enterprises, Inc. (71-379)			
VA	Animal Production		14,000
Laboratory Supply Company (71-641)			
VA	Animal Production		83,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Laboratory Supply Company (71-696)			
VA	Animal Production		$ 8,000
Laboratory Supply Company (70-2041)			120,000
VA	Animal Production	90,000	
VC	Trust Stocks & Genetic Control	30,000	
Little, Arthur D., Inc. (65-61)			1,625,000
IIC	Natural Product Assay	50,000	
IIIA	Primary In Vivo Screening	200,000	
IIIB	In Vitro Screening	30,000	
IIIC	Detailed Evaluation of New & Old Drugs	181,000	
IIID	Toxicology and Pharmacology	251,000	
VD	Tumor and Cell Culture Banks	38,000	
VIIA	Screening R&D	290,000	
VIIC	Pharmacology & Physiological Disposition	585,000	
Litton Industries, Inc. (68-1287)			
IVA	Patient Supportive Care		21,000
Makerere University (67-1343)			
IVB	Clinical Experimental Therapeutics		184,000
Marshall Research Animals, Inc. (71-378)			
VA	Animal Production		13,000
Mason Research Institute (70-2055)			399,000
IIID	Toxicology and Pharmacology	299,000	
VIA	Data Processing	100,000	
Mayo Foundation (70-2066)			
IVB	Clinical Experimental Therapeutics		(126,000)[1/]
Medical College of Virginia (69-2266)			
IIID	Toxicology and Pharmacology		6,000
Merck and Company (62-479)			449,000
IA1	Clinical Preparative Laboratories	230,000	
IA2	Non-Clinical Preparative Laboratories	219,000	
Miami, University of (70-2225)			
IIIB	In Vitro Screening		59,000
Michigan Department of Health (65-7)			
IIC	Natural Product Assay		35,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Michigan, University of (68-1284)			
IC	Dosage Formulation		$ 69,000
Michigan, University of (69-2004)			
IB	New Synthesis		39,000
Microbiological Associates, Inc. (68-1283)			1,944,000
IB	New Synthesis	272,000	
ID	Quality Control, etc.	174,000	
IIIA	Primary Screening	60,000	
IIIC	Detailed Evaluation of New & Old Drugs	335,000	
IIID	Toxicology & Pharmacology	157,000	
VA	Animal Production	338,000	
VD	Tumor and Cell Culture Banks	25,000	
VIIA	Screening R&D	224,000	
VIIC	Pharmacology & Physiological Disposition	224,000	
VIID	Immunity & Immune Response	135,000	
Microbiological Associates, Inc. (69-2177)			
IVA	Patient Supportive Care		51,000
Midwest Research Institute (65-94)			
IB	New Synthesis		275,000
Midwest Research Institute (69-2113)			
ID	Quality Control, etc.		124,000
Monsanto Research Corporation (65-1044)			129,000
IA1	Clinical Preparative Laboratories	120,000	
IA2	Non-Clinical Preparative Laboratories	9,000	
Monsanto Research Corporation (67-658)			
IA1	Clinical Preparative Laboratories		147,000
National Academy of Sciences (64-44) (Task Order #6)			
VB	Animal Disease Control		15,000
National Academy of Sciences (64-44) (Task Order #16)			
VIC	Research Services (Miscellaneous)		20,000
National Bureau of Standards (69-49)			
IVC	Clinical Trials (Miscellaneous)		80,000
National Drug Co. (70-2227)			
IC	Dosage Formulation		(137,000)1/

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
New Jersey College of Medicine & Dentistry (66-52)			
VB	Animal Disease Control		$ 100,000
New York University (67-1174)			
IVB	Clinical Experimental Therapeutics		10,000
Ohio State University (67-1175)			
IVB	Clinical Experimental Therapeutics		10,000
Pittsburgh, University of (67-1186)			
ID	Quality Control, etc.		9,000
Pomona College (69-2112)			
VIIE	Research & Development (Miscellaneous)		31,000
Public Health Service Division of Federal Health Program Service (Transfer 64-3)			
IVB	Clinical Experimental Therapeutics		1,350,000
Rawley Farms (71-654)			
VA	Animal Production		80,000
Rawley Farms (71-669)			
VA	Animal Production		40,000
Research Triangle Institute (69-2019)			135,000
IIB	Plant & Other Natural Prod. Coll. & Iso.	120,000	
IIC	Related Assay Testing	15,000	
Rochester, University of (69-39)			
VIIC	Pharmacology & Physiological Disposition		80,000
Scientific Literature Corporation (69-2114)			
VIB	Literature, Publications & Related Activities		(111,000) 1/
Scientific Literature Corporation (70-2267)			
VIB	Literature, Publications & Related Activities		69,000
Simonsen Laboratories, Inc. (65-55 and 71-2101)			131,000
VA	Animal Production	32,000	
VC	Trust Stocks & Genetic Control	99,000	
Simonsen Laboratories, Inc. (71-639)			
VA	Animal Production		94,000

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Simonsen Laboratories, Inc. (71-657)			
VA	Animal Production		$ 48,000
South Shore Analytical & Research Laboratory, Inc. (64-932)			
IIID	Toxicology and Pharmacology		180,000
Southern Animal Farms (71-640)			
VA	Animal Production		81,000
Southern Animal Farms (71-645)			
VA	Animal Production		44,000
Southern Research Institute (71-2021)			
IB	New Synthesis		426,000
Southern Research Institute (71-2098)			1,387,000
IIIA	Primary In Vivo Screening	200,000	
IIIB	In Vitro Screening	34,000	
IIIC	Detailed Evaluation of New & Old Drugs	373,000	
VIA	Data Processing	25,000	
VIIA	Screening R&D	600,000	
VIIB	Biochemical Mechanisms	55,000	
VIIC	Pharmacology & Physiological Disposition	100,000	
Southern Research Institute (65-654)			
VIIC	Pharmacology & Physiological Disposition		170,000
Southern Research Institute (66-29)			
VIIB	Biochemical Mechanisms		271,000
Southern Research Institute (70-2083)			
IIID	Toxicology and Pharmacology		314,000
Stanford Research Institute (71-2070)			504,000
IB	New Synthesis	281,000	
ID	Quality Control, etc.	223,000	
Stanford Research Institute (69-37)			
IA2	Non-Clinical Preparative Laboratories		(60,000)[1/]
Starks Associates, Inc. (62-484)			169,000
IA1	Clinical Preparative Laboratories	85,000	
IA2	Non-Clinical Preparative Laboratories	84,000	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Starks Associates, Inc. (68-1275)			
IA2	Non-Clinical Preparative Laboratories		$ 70,000
Taconic Farms, Inc. (71-651)			
VA	Animal Production		67,000
Texas Inbred Mice Company (68-88)			242,000
VA	Animal Production	133,000	
VC	Trust Stocks & Genetic Control	109,000	
Texas Inbred Mice Company (71-658)			
VA	Animal Production		90,000
Texas, University of (66-1156)			
VIIC	Pharmacology & Physiological Disposition		198,000
Texas, University of (67-1143)			
IIID	Toxicology and Pharmacology		10,000
Thompson, J.I., Company (69-2158)			
VIA	Data Processing		22,000
TRW, Inc. (63-622) (formerly Hazleton Laboratories)			385,000
IIC	Natural Product Assay	99,000	
IIIA	Primary In Vivo Screening	223,000	
IIIC	Detailed Evaluation of New & Old Drugs	59,000	
VIIA	Screening R&D	4,000	
TRW, Inc. (66-84) (formerly Hazleton Laboratories)			
VIIA	Screening R&D		316,000
TRW, Inc. (68-1286) (formerly Hazleton Laboratories)			
IVA	Patient Supportive Care		396,000
TRW, Inc. (69-2067) (formerly Hazleton Laboratories)			
IIID	Toxicology & Pharmacology		36,000
Tulane University (65-593)			85,000
VA	Animal Production	17,000	
VC	Trust Stocks & Genetic Control	68,000	
Upjohn Company (68-1023)			500,000
IIA	Antibiotic Fermentation & Isolation	119,000	
IIC	Natural Product Assay	25,000	
IIIB	In Vitro Screening	73,000	

TABLE III (Continued)

CONTRACTOR	FUNCTIONS		DOLLAR LEVEL
Upjohn Company (68-1023) cont.			
VIIA	Screening R&D	50,000	
VIIB	Biochemical Mechanisms	112,000	
VIIC	Pharmacology & Physiological Disposition	121,000	
Utah, University of (65-1041)			
IB	New Synthesis		$ 105,000
Veterans Administration (Transfer 64-4)			
IVB	Clinical Experimental Therapeutics		1,029,000
Virginia, University of (71-2099)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		93,000
Vitro Laboratories (69-58)			
VIA	Data Processing		453,000
Walter Reed General Hospital (Transfer 64-5)			
IVB	Clinical Experimental Therapeutics		49,000
WARF Institute, Inc. (66-494)			303,000
IIC	Natural Product Assay	98,000	
IIIA	Primary In Vivo Screening	154,000	
IIIC	Detailed Evaluation of New & Old Drugs	46,000	
VIIA	Screening R&D	5,000	
WARF Institute, Inc. (67-747)			
IIB	Plant & Other Natural Prod. Coll. & Isolation		42,000
Wisconsin, University of (67-1126)			
IIC	Natural Product Assay		15,000
Yale University School of Medicine (67-1193)			15,000
IIIB	In Vitro Screening	12,000	
VIIC	Pharmacology & Physiological Disposition	3,000	
Yale University School of Medicine (70-2089)			
IIID	Toxicology and Pharmacology		90,000

1/ Not included in dollar totals since no Fiscal Year 1971 funds were obligated; included here for reference since it is an on-going contract.

TABLE IV
DESCRIPTION OF CONTRACTS
IN THE CHEMOTHERAPY PROGRAM

ABCOR, INC. (NIH-70-2239)

The object of this effort is to improve the efficiency of granulocyte procurement using the NCI-IBM Cell Separator.

AEROJET-GENERAL CORPORATION (PH43-68-1300)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

AGRICULTURE, U.S. DEPARTMENT OF (TRANSFER OF FUNDS)

Under the transfer of funds agreement with the U.S.D.A. the New Crops Research Division collects approximately 1,000 new plant materials per year for screening. Plant samples are obtained from all parts of the world.

The plants collected by the U.S.D.A. are shipped to the extraction contractor, WARF Institute, Inc. As confirmed active plants appear, the U.S.D.A. recollects in large quantity, on a priority based on the needs of the Chemotherapy Program, at the rate of about 100 recollections per year for shipment to the fractionating chemists.

AMERICAN CANCER SOCIETY (NIH-71-743)

Support is provided for a conference on clinical staging of Hodgkin's disease.

AMERICAN NATIONAL RED CROSS (NIH-70-2005)

The Red Cross supplies the Medicine Branch and other areas of the National Cancer Institute with 120-150 units of platelet concentrates (fresh) per week for experimental use in the clinical wards.

ANTEC CORPORATION (NIH-71-380)

This procurement contract provides 150 beagles for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

ARIZONA, UNIVERSITY OF (PH43-67-1484)

The major objective of this contract is the fractionation of confirmed active plants in order to isolate in a pure state and identify the anti-tumor active components. Recent promising results have been obtained from the extraction of plants collected in Greece, Hawaii and Arizona. Further fractionation is being carried out to isolate the active components.

ARS/SPRAGUE-DAWLEY (NIH-70-545)

This procurement contract supplied 41,700 Fischer 344 rats yearly for rodent tumor tests. Original breeding animals were derived by hysterotomy and isolator techniques in order to reduce disease levels by eliminating those of major importance. This contract terminated December 31, 1970.

ARS/SPRAGUE-DAWLEY (NIH-2007)

This genetic-production center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in closely controlled environments. The overall workscope comprises four distinct segments identified as follows:

Segment A provides inbred, outbred and hybrid rodents in modified conventional environments.

Segment B provides Syrian golden hamsters with defined but non-pathogenic organisms. A small percentage of the effort is devoted to the development of germfree hamsters and gerbils.

Segment C provides inbred and outbred laboratory strains of rats as germ-free and/or with defined microflora.

Segment D provides various germfree inbred, outbred and hybrids of inbred rodents; and the production of counterparts of the various species with defined microflora.

ARS/SPRAGUE-DAWLEY (NIH-70-2044)

This rodent breeding center contract supports a production effort designed to furnish approximately 60,000-70,000 animals annually. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

ARS/SPRAGUE-DAWLEY (NIH-71-650)

This procurement contract provides 156,000 BDF_1 (C57/BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

ASSOCIATED BIOMEDIC SYSTEMS, INC. (NIH-70-2271)

The contractor provides gram quantities of various hematopoietic cell lines in tissue culture for biochemical studies relating to tRNA and the RNA dependent DNA polymerase.

ATOMIC ENERGY COMMISSION (NIH-71-56)

The principal objective of this research project is to determine the feasibility of screening new agents for their ability to inhibit cells in G_1 arrest. Studies are aimed at the development of an experimental model for such screening based on the contractor's established method for (a) the reversible arrest of viable cells in the G_1 phase of cell cycle and (b) the release of G_1 arrested cells permitting progression into the S-phase. Should the feasibility of the method for screening be established, the contractor will formulate and submit appropriate protocols for use by Chemotherapy in larger-scale contract projects.

BATTELLE MEMORIAL INSTITUTE (NIH-69-2251)

The Contractor maintains a genetic-production center for inbred rodents which provides breeding animals for large-scale laboratory rodent production colonies and limited numbers for testing and research programs. The profile of categories of these animals includes ten strains.

BATTELLE MEMORIAL INSTITUTE (NIH-70-2008)

The Contractor performs bacteriologic diagnostic services with emphasis upon the detection of Salmonella spp. and Pseudomonas spp. in program rodents, and other materials used for animal husbandry practices. Specimens are forwarded by contract laboratories and animal production establishments. Approximately 10,000 samples are screened per year. The research portion of this contract (a minor component) is directed towards improvement in cultural techniques and the elucidation of salmonella inhibitory substances associated with certain microorganisms.

BELLAIRE ACRES (NIH-71-649)

This procurement contract provides for the supply of 156,000 BDF_1(C57BL/6♀ x DBA/2♂) hybrid mice yearly for the CCNSC compound evaluation program. Breeding animals originate in genetic centers and/orC57BL/6 and DBA/2 production colonies.

BELLAIRE ACRES (NIH-71-652)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

BEN VENUE LABORATORIES, INC. (PH43-66-909)

This contract is designed to provide a pharmaceutical pilot plant for the development, formulation and production of parenteral clinical dosage forms of antitumor agents. Ben Venue Laboratories has the capacity for preparing large-scale production batches of dry-filled, liquid-filled and lyophilized sterile parenteral products. Specifically, the contractor performs the following duties:

1. Solubility and stability studies on chemical agents prior to development of a specific dosage form;

2. Development and production of parenteral dosage forms for clinical evaluation;

3. Stability surveillance of all dosage forms prepared.

BIONETICS RESEARCH LABORATORY (NIH-71-2057)

This is a contract for procurement of relatively large amounts of normal and neoplastic tumor cells in tissue culture. Tumor cells with variable growth rates are provided as scheduled by the project officer for investigation of biological and biochemical parameters underlying cell growth and neoplastic transformation.

BOWMAN GRAY SCHOOL OF MEDICINE (PH43-68-950)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

BRISTOL LABORATORIES (NIH-69-35)

This contract covers a comprehensive effort to obtain new antitumor agents from fermentation broths or other natural products. It includes (1) the preparation of broths, or other natural products, for screening against *in vivo* systems specified by NCI, (2) a rodent tumor laboratory for *in vivo* screening and also testing of fractionation and other follow-up studies, (3) the isolation work required to obtain purified agents, (4) development and use of *in vitro* correlative assays for more rapid follow-up of such samples, (5) production of large quantities of agents approved for clinical trials, and (6) the preparation of appropriate dosage forms of such agents.

CALIFORNIA, UNIVERSITY OF (NIH-70-2014)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance

CALIFORNIA, UNIVERSITY OF (NIH-70-2014) (Continued)

with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

CALIFORNIA, UNIVERSITY OF (NIH-70-2092)

This contractor provides research support for the more complex problems in tissue typing (see PH43-69-2177) in addition to furnishing typing plates and specific antisera. He also makes available to investigators in NCI typing data on a very large population of random donors.

CENTRAL DRUG RESEARCH INSTITUTE (PL-480 - Agreement No. NIH-01-004-1)

The Central Drug Research Institute, Lucknow, India, collects native Indian medicinal plants, supplies extracts for screening by CCNSC, and fractionates the active extracts. During the past two years the budget was increased to provide for (1) synthesis of compounds of chemotherapeutic interest, and (2) setting up an antitumor screening laboratory to provide in-house testing capability. Several compounds have been received by CCNSC and limited in-house testing has been achieved.

CHARLES RIVER BREEDING LABORATORIES, INC. (PH43-66-87)

This contract has as its objectives the development of germ-free foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in jacket isolators. Offspring from this second stage are issued to secondary genetic centers, which in turn produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators. A colony of agoutis (Dasyprocta agouti) is maintained in order to furnish serum for L-asparaginase extraction.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-443)

This procurement contract supplies 41,000 Fischer 344 rats yearly for rodent tumor tests. Original breeding animals are derived by hysterotomy and isolator techniques in order to reduce disease levels by eliminating those of major importance.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-668)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice free of pseudomonads for CCNSC contract

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-666) (Continued)

studies. The breeding animals, which originate from the Genetic Center at this site, are from the isolator system and are free from parasites and pathologic organisms.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-70-2043)

This rodent breeding center contract supports a production effort designed to furnish approximately 60,000-70,000 animals annually. Breeding animals are furnished by the Government. Major emphasis is directed toward AKR mouse production.

CHARLES RIVER BREEDING LABORATORIES, INC. (NIH-71-644)

This procurement contract provides for the supply of 78,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for CCNSC compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

CHEMICAL ABSTRACTS SERVICE (PH43-65-1021)

The Chemical Abstracts Service (CAS) is operating an automated NCI chemical information system similar to the CAS Registry System. The data bank contains information on about 157,000 unique compounds submitted to CCNSC for screening and on other selected compounds of interest. Included is such information as the chemical structure representation, corresponding substructure fragments, and CAS Registry Numbers for NCI compounds. The magnetic tape files are used to determine which newly-offered compounds duplicate those already in the screen, to carry out substructure searches for analogs of compounds of interest, and to prepare formatted magnetic tapes of name and molecular formula cross-referenced to NSC number ready for translation and printing at DCRT's computer facility. Full structure searches are made of large offerings from other collections to identify nonduplicates and thereby facilitate the acquisition of new materials for screening. Some consultation in nomenclature and system analysis is also provided.

CHILDREN'S HOSPITAL OF L.A. (NIH-70-2020)

This contract provided for field testing in granulocyte procurement of the NCI-IBM Cell Separator. The principal investigator was able to perform only a few runs and it was expedient to recall the Cell Separator for use elsewhere in the Chemotherapy Program. This contract terminated December 3, 1970.

COLLABORATIVE RESEARCH, INC. (PH43-66-506)

The major objective of this contract is the production of homofolic acid (NSC-79249) and tetrahydrohomofolic acid (NSC-89473) in kilogram quantities.

COLLABORATIVE RESEARCH, INC. (PH43-66-506) (Continued)

Tetrahydrohomofolic acid is needed in significant quantities for pre-clinical pharmacological evaluation. Development work on the large-scale preparation of these substances has also been done under this contract. In addition, the synthesis of some analogs and derivatives of homofolic acid and cytoxan has been undertaken.

CONTROL DATA CORPORATION (PH43-67-1356)

Clinical patient research data files are being maintained and queried by use of INFOL (Information Oriented Language). The CDC 915 Optical Scanning capability is used for data capture. A brain tumor protocol study is also being analyzed.

CORNELL UNIVERSITY MEDICAL COLLEGE (NIH-69-60)

The purpose of this contract is to support the clinical use of asparaginase through basic studies of the nature of the biochemical changes which accompany tumor sensitivity and resistance to the enzyme, as well as investigations into the causes and prevention of undesirable side effects.

These objectives are to be accomplished in part through study of the chemical and physical aspects as well as the mechanism of action and the selection of inhibitors for the enzyme asparagine synthetase. A related area of study is the search for the identity of the protein or proteins whose limitation, as a result of asparagine deprivation, results in cell death.

COSTA RICA, UNIVERSITY OF (PH43-64-31)

The contract is concerned with the collection of native Costa Rican plants and preparation of extracts for testing by CCNSC.

Aqueous-alcoholic extracts of native plants, many with reputed medicinal properties, are prepared and sent for testing by CCNSC. Plants whose extracts possess reproducible activity are recollected in large quantity and shipped to one of our contract fractionators, where chemical isolation studies are undertaken.

During the current year new plants have been collected and about 150 extracts prepared, usually from two parts of each plant. Several large recollections have been made and shipped to the fractionator. In all, the fractionator has about a dozen large recollections with nearly all under fractionation.

DELAWARE, UNIVERSITY OF (NIH-69-2115)

Principal contract objectives are (1) to further purify an L-glutaminase from Aerobacter aerogenes for therapeutic and toxicologic testing; this

DELAWARE, UNIVERSITY OF (NIH-69-2115) (Continued)

amidohydrolase is active at physiological pH and is inert towards L-asparagine; (2) to determine the amino acid sequence of the active site of L-asparaginase from E. coli and selectively to modify that active site without destroying the catalytic integrity of the enzyme.

DUKE UNIVERSITY MEDICAL CENTER (NIH-70-2030)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

ELDER, PAUL B., COMPANY (NIH-69-2110)

This was a service contract for the development, formulation and production of tablets and capsules. The dosage forms produced by this contractor were packaged and labelled for distribution to chemotherapy investigators. This contract terminated October 31, 1970.

FLOW RESEARCH ANIMALS, INC. (NIH-71-655)

This procurement contract provides for the supply of 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for CCNSC compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

FLOW RESEARCH ANIMALS, INC. (NIH-71-656)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (NIH-71-2123)

The objective of this program is to conduct virologic studies of patients participating in the National Cancer Institute's chemotherapeutic program to make possible evaluations that will aid the clinician in the selection of measures for supportive care and allow for the development of information on the importance of the role of viruses in the cancer patient in both protected and conventional environments.

GEORGE WASHINGTON UNIVERSITY (NIH-69-2116)

The purpose of this project is to facilitate scanning and hard copy retrieval of cancer chemotherapy literature by incorporating into an automatic microfilm reader-printer system the documents and/or abstracts comprising those cited in Cancer Chemotherapy Abstracts, (CCA), Volume 1, 1960 through Volume 7, 1966, and, further, to provide both cumulative agent and author indexes.

GEORGIA INSTITUTE OF TECHNOLOGY (PH43-68-1288)

The objective of the contract is the total synthesis of the alkaloid, camptothecin (NSC-94600), and the preparation of analogs and derivatives of it. The approach is to devise a practical synthesis which would be potentially suitable for scale-up from commercially available starting materials. Camptothecin sodium salt (NSC-100880), which is prepared from camptothecin, is undergoing clinical evaluation and the supply of this alkaloid from natural sources is very limited.

HARLAN INDUSTRIES, INC. (NIH-71-646)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

HAWAII, UNIVERSITY OF (PH43-67-748)

This contract provided originally for the collection of a minimum of 500 plant samples from among the indigenous and exotic higher plants of the Hawaiian Islands and the recollection of larger samples of those that are reproducibly active in the CCNSC tests. Plants so obtained are shipped to the extraction contractor, WARF Institute, Inc., and the extracts tested at one of our contract screeners. During the current year 17 large recollections of confirmed active plants were made and sent to the fractionator. Since priority was given to recollections, the general collections were reduced to a very small number.

HEALTH RESEARCH, INC. (NIH-70-2035)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The Contractor carries out the service by: (1) performing iso-antigenic typing tests of tumors used in the various screening tests, enabling CCNSC to maintain and distribute tumors indentical to the prototype; (2) developing techniques and material for antigenic typing of tumors new to the

HEALTH RESEARCH, INC. (NIH-70-2035) (Continued)

screening program; (3) determining strain specificity of tumors originating in the inbred mouse; and (4) performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic-production centers.

HORTON LABORATORY ANIMALS, INC. (NIH-71-684)

This procurement contract provides for the supply of 78,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for CCNSC compound evaluation studies. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

HORTON LABORATORY ANIMALS, INC. (NIH-71-709)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. The breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

IIT RESEARCH INSTITUTE (PH43-67-1141)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of two potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

IIT RESEARCH INSTITUTE (PH43-68-1021)

The primary purpose of this contract is to provide for the performance of *in vivo* testing of new synthetic materials and natural products, and also for animal health and utilization studies assigned specifically for problems that arise in various screening laboratories and animal supply contractors. Screening is being conducted according to CCNSC protocol at a level of approximately 19,000 tests per year. The lymphoid leukemia L1210 in the mouse host is utilized for all synthetics and new natural products are being tested in both the L1210 and the P388 leukemia in the mouse host. The Walker 256 carcinosarcoma in the rat host continues to be used for the follow-up of natural products previously found active in this system if

IIT RESEARCH INSTITUTE (PH43-68-1021) (Continued)

they do not have correlative activity in any other system. Testing in the Walker 256 tumor test system has dropped to a negligible level. Studies with the AKR mouse leukemia have been initiated this year and an increased effort with this system is planned. The assay systems used, however, are determined by the requirements of the Chemotherapy Program, NCI, and may be changed when specifically requested by the Project Officer.

As a second function, this laboratory also maintains a routine Salmonella spp. and Pseudomonas spp. diagnostic laboratory and provides general animal disease diagnostic services as requested. These studies include virology, bacteriology, pathology and histopathology. In addition, it includes base line studies to define the microbiological flora and virus contact status of various species of interest to the program. This portion of the contract provides the quality controls necessary for the maintenance of the quality of the animals utilized in the CCNSC program.

Methodology or research and development is carried out only at the request of the Project Officer and during this year has been devoted to answering specific questions that arose in the in vivo screening and animal health and utilization programs.

INDIAN CANCER RESEARCH CENTRE (PL-480 - Agreement No. NIH-01-010-1)

This agreement provides for the collection and identification of Indian plants, extraction and preparation of crude extracts, and for the continued operation, in Bombay, India, of an in vivo screening laboratory for testing materials, both natural products and synthetics, available in India. A mouse breeding colony is maintained for animal production. Testing is conducted according to CCNSC protocols with the lymphoid leukemia L1210 and the lymphocytic leukemia P388 test systems being utilized currently.

INDIANA UNIVERSITY FOUNDATION (NIH-70-2028)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

INFORMATION AND COMMUNICATIONS APPLICATIONS, INC. (NIH-69-2236)

The purpose of this contract was to extend the clinical data processing system developed by the Program Analysis Branch by designing a data management system for hematologic, bacteriologic, immunologic, clinical responses and survival data. The immediate goal of this system was to process a variety of experiments involving blood cell separators and leukocyte infusions. The file structures were designed to be compatible with other

INFORMATION AND COMMUNICATIONS APPLICATIONS, INC. (NIH-69-2236) (Continued)

PRA data banks. Aid in designing the forms to implement this system was provided by the Contractor. Files were built using a large number of retrospective cell collections and a few recent ones. This contract terminated January 1, 1971.

JACKSON LABORATORY (NIH-71-80)

This service contract provides for the annual procurement of approximately 31,200 C57BL/6 and 15,600 DBA/2 inbred mice to screening contractors for tumor transplantation and as breeders for large-scale hybrid production colonies. It also supplies 49,400 AKR inbred mice for compound evaluation studies. Breeding animals are supplied by the Contractor.

JEFFERSON MEDICAL COLLEGE (NIH-70-2136)

The principal objective of this contract is to obtain information on the absorption, distribution, metabolism, and excretion of antitumor agents undergoing Phase I clinical trial in patients. Quantitative methodology is developed where necessary for the measurement of a particular agent and its metabolite(s) in blood, cerebrospinal fluid, bile, urine, and tissue specimens. Limited preclinical drug disposition studies are performed in laboratory animals to correlate with and supplement experimental observations made in patients. During the past contract year, the research effort was primarily directed toward the accrual of clinical pharmacological data on dibromodulcitol (DBD) and preliminary studies were conducted with streptozotocin. Results of these studies are integrated into the NCI Chemotherapy Program effort to obtain basic quantitative data on drug disposition which will permit design of drug dosage and scheduling requirements for obtaining maximum therapeutic effect in patients with malignant disease.

KANSAS, UNIVERSITY OF (NIH-71-2010)

This Contractor maintains a genetic-production center. Its most important function is to secure the maintenance of the strains of rodents that are of interest in CCNSC program investigations. Its spectrum includes six strains. Small groups of animals in excess of pedigree requirements are shipped to contractor laboratories for developmental studies. These strains include C57BL/6, DBA/2, AKR, BALB/c, C3H/He and A/He. Animals not required for perpetuation of pedigree are supplied to screening contractors and as breeders for production colonies.

KENTUCKY, UNIVERSITY OF (PH43-67-1173)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

LABORATORY RESEARCH ENTERPRISES, INC. (NIH-71-379)

This procurement contract provides 150 beagles for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

LABORATORY SUPPLY COMPANY (NIH-71-641)

This procurement contract provides for the supply of 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for the CCNSC screening program. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

LABORATORY SUPPLY COMPANY (NIH-71-696)

This procurement contract is designed to furnish 13,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) mice free of pseudomonads for CCNSC contract studies. Breeding animals are furnished by the Government. Water for drinking and equipment washing is chlorinated at a level of 10 ppm. This contract will terminate June 1, 1971.

LABORATORY SUPPLY COMPANY (NIH-70-2041)

This Contractor maintains a genetic trust stock colony of six inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

LITTLE, ARTHUR D., INC. (PH43-65-61)

This is a broad-based major research and development program which has been carefully tailored to the overall mission of the NCI Chemotherapy Program. The task objectives range from routine screening activities, in prescribed in vivo and in vitro systems, to highly specialized exploratory research on the physiological disposition of active agents, neuropharmacological studies, including the development of new methodologies, central nervous system solid tumor studies, etc. The total program is divided into nine major categories, each of which is closely coordinated by a specially selected Assistant Project Officer from the appropriate NCI Chemotherapy Program area.

The Screening and Experimental Therapeutics division is basically concerned with the routine screening, in in vivo systems, of synthetic and natural products, as well as companion studies on route and regimen, the effectiveness of combination chemotherapy, and efforts to develop new and improved screening techniques.

LITTLE, ARTHUR D., INC. (PH43-65-61) (Continued)

The Cell Kinetics studies include (1) investigations of hematological recovery in the bone marrow of normal drug-treated mice, for the purpose of selecting optimal therapy regimens and for the possible detection of delayed toxicity for the host, (2) measurement of generation times, doubling times and mitotic indices of solid and ascitic tumors via various techniques, (3) bioassay studies with L5178Y lymphocytic leukemia, and (4) related methodological studies.

The Preclinical Toxicology portion is addressed to routine and special pharmacology and toxicology studies in response to problems which arise in the preclinical and clinical phases of the Chemotherapy Program, together with methodological studies directed to the development of related new methodologies.

The Physiologic Disposition and Biochemistry phase of the effort provides for sophisticated exploratory research on the mode of action and physiological disposition of active agents, and includes a cooperative project with selected clinical groups to measure the metabolic fate and physiologic disposition of drugs being employed in the management of cancer in humans.

The Neuropharmacology studies are aimed at approaching various toxicological and pharmacological problems relating to the treatment of neoplastic diseases of the central nervous system. Improved techniques are being sought for the administration of agents used to control neoplastic disease in the CNS.

The Central Nervous System solid tumor studies are concerned with the development and evaluation of transplantable gliomas in the CNS of Beagle dogs, together with collaborative studies with clinical and pharmacology groups of the NCI to examine the growth kinetics and the behavior of transplantable gliomas to selected agents.

The Cell Culture and Methodology work consists of (1) routine cell culture testing of synthetic compounds, antibiotic filtrates and other natural products according to standard protocols, and (2) special studies for the development of improved methods of studying the influence of drugs on cell growth kinetics. The Contractor employs the spin filter continuous culture techniques developed by this group for the study of the mechanisms associated with cell kill, mode of action and cell uptake of selected antitumor agents, etc.

The Electron Microscopy and Radioautography program provides such services in support of the pharmacology, toxicological, microbiological and solid tumor studies.

The Cryobiology and Tumor Distribution activities provide for the maintenance of a frozen tumor and cell bank, distribution of standard tumor lines to screening laboratories and other research institutions, and methodological studies on the frozen-stored tumor lines.

LITTON INDUSTRIES, INC. (PH43-68-1287)

This contract originally provided for the development, fabrication and improvement of experimental patient isolator units for patients undergoing intensive chemotherapy in NCI sponsored programs. Four portable prototype laminar flow units have been installed in the Clinical Center for initial clinical trials. Additional units have been installed at the Baltimore Cancer Research Center and at the NCI-VA Medical Oncology Unit at the Washington, D. C. VA Hospital. The contractor now conducts intensive microbiological surveillance of patients in the protected environment studies.

MAKERERE UNIVERSITY (PH43-67-1343)

This contract supports the Lymphoma Treatment Centre at Makarere University, Kampala, Uganda under the immediate supervision of Dr. John Ziegler. A large group of patients with Burkitt's tumor are available in the area. Studies are progressing in Burkitt's tumor patients, evaluating their response to therapy; immune host factors and metabolism of drugs in African patients. Studies in patients with solid tumors including melanoma, Kaposi's sarcoma and hepatocellular carcinoma are in progress with emphasis on experimental chemotherapy and clinical pharmacology.

MARSHALL RESEARCH ANIMALS, INC. (NIH-71-378)

This procurement contract provides 150 beagles for toxicologic assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the Contractor.

MASON RESEARCH INSTITUTE (NIH-70-2055)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of 13 potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

MAYO FOUNDATION (NIH-70-2066)

The purpose of this contract is the investigation of new drugs in the treatment of advanced gastrointestinal tumors as a cooperative venture between The Mayo Foundation and the National Cancer Institute. The objective is to have each drug evaluated in a minimum of 20 cases of adenocarcinoma of the

MAYO FOUNDATION (NIH-70-2066) (Continued)

colon to meet the requirements of the chemotherapy program linear array. Provision has been made for reporting of data on each trial in a manner which is consistent with the data retrieval currently employed intramurally at NCI. This will enable both direct comparisons and possible integration of data on new drugs from both of these sources.

MEDICAL COLLEGE OF VIRGINIA (NIH-69-2266)

This contract is concerned with testing bacterial endotoxins of E. coli and Salmonella and exotoxins of Streptococcus and Staphylococcus species for possible synergistic toxicity with selected cancer chemotherapeutic agents. Antidotes for the synergistic toxicities are being sought.

MERCK AND COMPANY (PH43-62-479)

This contract provides for the resynthesis of compounds in the amounts needed for clinical trial or for necessary preclinical evaluations. About 50% of the budget is devoted to clinical preparations.

This is the largest and oldest of the CCNSC preparative laboratories. Because of their extensive and diverse experience, the Contractor is able to prepare compounds of many different and difficult types. These are materials for which no commercial source is available or which cannot be prepared in the required quantities by the original supplier.

MIAMI, UNIVERSITY OF (NIH-70-2225)

This contract provides for the in vitro cell culture presumptive screening of plant materials, and the follow-up testing of natural products having demonstrated correlative activity in the CCNSC in vivo screening program, as a tool for fractionation. Presumptive screening of synthetic materials is also carried out when the quantity of these materials is not sufficient to do primary in vivo screening. The procedures are those prescribed by CCNSC protocols which are based on the methods established by Eagle and Foley. Approximately six thousand tests at three to five dose levels per test are performed yearly.

MICHIGAN DEPARTMENT OF HEALTH (PH43-65-7)

This contract provides assay services, utilizing a variety of cell culture methods, as aids in the purification of potential antitumor agents from fermentation broths. The methods employed include paper chromatography and paper electrophoresis in conjunction with cell culture agar diffusion bio-autography, as well as growth inhibition assays, both in stationary and suspension cultures. The related fermentation and isolation program, serviced by this assay contract, is financed by the Michigan Department of Health.

MICHIGAN, UNIVERSITY OF (PH43-68-1284)

The primary objectives of this contract are two-fold: (1) the development of parenteral clinical dosage forms of potentially active antineoplastic agents, and (2) fundamental research in seeking out new approaches in the formulation of compounds of limited solubility and/or stability. Part 1 of the contract relates to immediate formulation problems and Part 2 involves longer range studies on new concepts of dosage formulation.

MICHIGAN, UNIVERSITY OF (NIH-69-2004)

This contract is for the chemical synthesis of a number of new nitrogen heterocyclic compounds containing complex ring systems. The compounds are for primary screening and are unique structures which have been little explored and/or are heretofore unknown. All materials are fully characterized, of high purity and in sufficient quantity for adequate evaluation. Recent effort has been directed towards the total synthesis of camptothecin, a drug which is in clinical trial.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-68-1283)

This particular program is the product of the realignment and gradual consolidation, over a number of years, of individual contracts with this Company (both service and research oriented) which now forms a single broad-based coordinated mechanism for the detailed investigation of problems which arise in the chemotherapy of malignant disease. The total program is closely integrated scientifically with NCI operations, and the activities are carried out under the daily guidance of professional staff of the CCNSC and Experimental Therapeutics area of the NCI Chemotherapy Program. Projects selected for study are those which are determined to be highly pertinent to the needs of the preclinical and clinical programs of the NCI. The contract is divided into eight basic divisions, each of which is closely monitored by an Assistant Project Officer.

Chemotherapy Research - Activities in this Division involve exploratory research in biological and biochemical areas of chemotherapy, and include studies on cell kinetics, the search for and evaluation of new screening methods, studies related to remission induction and maintenance, etc.

Drug Evaluation - This Division is concerned with the quantitative evaluation of the therapeutic effectiveness of drugs, including studies on the optimal conditions for administering both old and new drugs, separately and in combination. The program also includes the conduct of primary screening tests at a rate commensurate with the needs of the CCNSC screening effort. In addition, this Division evaluates and applies the utility of new methodologies developed collaboratively with the Chemotherapy Research Division.

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-68-1283) (Continued)

Immunology - Investigations in this Division involve basic studies on the immunology of viral oncogenesis which may lead to a better understanding of the means for controlling or suppressing the disease through immunology alone or in combination with chemotherapy.

Toxicology - The toxicology portion of this contract is directed toward: (1) the study of the effect of oncolytic agents on the cardiovascular system, (2) the mechanism of cardiotoxicity, and (3) development of methods for the prevention or modification of the cardiac effects.

Drug Metabolism - This group functions as a source for the study of the physiological distribution and metabolism of antitumor drugs, with special attention to their relationship to therapeutic effectiveness and toxicity.

Organic Chemistry - This portion of the contract is directed to the synthesis of new compounds of potential usefulness, the examination of chemical problems of drug development, and studies on the structure of new agents.

Drug Distribution - This Division provides services to the NCI for the storage and distribution of drugs and chemicals to various research laboratories throughout the country.

Animal Husbandry - The function of this Division is primarily the operation of a genetic center for mouse strains needed in support of the research activities of the contract. Holding facilities and animal caretaking services are a major function of this project.

MICROBIOLOGICAL ASSOCIATES (NIH-69-2177)

The contractor performs routine histocompatibility tissue typing of patients in NCI and potential donors of leukocytes, platelets, or bone marrow.

MIDWEST RESEARCH INSTITUTE (PH43-65-94)

Midwest Research Institute is one of three major synthetic contractors for the CCNSC. The primary objective of this contract is the rapid exploitation of leads generated by the Drug Evaluation Branch and clinical groups. This Contractor has provided a large number of compounds to CCNSC for screening. These compounds represent the exploration of a variety of leads. Current investigation centers around three areas:

1. Chemical extension of clinically significant materials: structural modification of folic acid and related compounds; structural variations of natural quinones; synthesis of bleomycin analogs; and synthesis of camptothecin and other natural products.

2. Analogs of biologically active agents: structural modifications of pyrazine N-oxides; tropolones; polyamines; pyrazole; hydroxamic acids; 2,4'-bithiazoles; and thiazoles.

MIDWEST RESEARCH INSTITUTE (PH43-65-94) (Continued)

3. Extracts from Chinese medicinal plants are being prepared for antitumor evaluation.

The materials prepared have been of high purity, thoroughly characterized and in sufficient quantity for adequate evaluation.

MIDWEST RESEARCH INSTITUTE (NIH-69-2113)

Midwest Research Institute is one of the two facilities where analyses of experimental medicinal compounds and their clinical formulations are performed for the CCNSC. The Contractor determines the identity and purity of these materials developing procedures for their analysis when standard methods are not available. The techniques employed include elemental analysis, chromotographic and spectrometric procedures, and other physiochemical procedures appropriate to the situation.

MONSANTO RESEARCH CORPORATION (PH43-65-1044)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at Monsanto or acquired from other sources, are analyzed for purity and identity by radioautography, assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the NCI staff.

MONSANTO RESEARCH CORPORATION (PH43-67-658)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not available on the open market or from the original supplier in the amounts required. The major effort of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities. This contract also provides for the large-scale extraction, isolation, and purification of natural products required for preclinical and clinical studies.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #6)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals; standards for nomenclature used to identify stocks and strains of laboratory animals; standards for methodology in maintaining axenic and gnotobiotic animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL ACADEMY OF SCIENCES (PH43-64-44 - TASK ORDER #16)

This contract Task Order provides for conferences on diverse subjects that are intimately associated with the field of bio-medical research. The agenda for specific meetings may be concerned with a common problem, or to develop the basis for a research program that is being considered by a segment of the scientific community. Conferences in the past have been held on research facilities; diseases of laboratory animals; professional training; the provision of animals for biological models for research; gnotobiotic technology; and requirements for standards in specific areas. Speakers are invited to present their thoughts, and time is provided for open forum discussions. The proceedings of these conferences are published and made available to the scientific community.

NATIONAL BUREAU OF STANDARDS (NIH-69-49)

This interagency agreement provides for a collaborative NCI-NINDB-NBS study on automatic identification of cells and counting of radioautographic grains. A computer-controlled scanning optical microscope and a library of subprograms have been developed and will be used to experiment with different strategies for discriminating among cell types.

NATIONAL DRUG COMPANY (NIH-70-2227)

This contract provides a facility for the development and production of oral and parenteral dosage forms with emphasis on injectable products. The National Drug Company is capable of developing sophisticated dosage forms and producing large-scale production lots of oral and parenteral products. All formulated lots are evaluated by the contractor for shelf life stability.

NEW JERSEY COLLEGE OF MEDICINE AND DENTISTRY (PH43-66-52)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, SV5, Toolan's H-1, and the Rauscher and Moloney viruses. The Contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; antibody-producing capacity of tumor-bearing mice and the effect of the route of injection of antibody levels; and a comparison of the sensitivity of caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 50,000 rodent serum and 3,000 tumor fragment diagnostic tests. The Contractor also prepares large quantities (40,000-50,000 ml. yearly) of vaccinia virus used for immunizing mice against infectious ectromelia.

NEW YORK UNIVERSITY MEDICAL CENTER (PH43-67-1174)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

NEW YORK UNIVERSITY (PH43-67-1336)

The purpose of this contract extension is to carry out analyses of data previously accumulated on patients having central nervous system neoplasms. The Contractor used and improved previously developed computer programs for analysis of all documents obtained to date. These documents include, but are not limited to, hospital discharge summaries, various diagnostic procedures (angiograms, brain scans, neuroradiology reports), and surgical pathology reports. This contract will terminate April 30, 1971.

OHIO STATE UNIVERSITY (PH43-67-1175)

The purpose of this contract is to provide data retrieval support for experimental chemotherapy of selected patients with brain tumor in accordance with the protocols of the Brain Tumor Chemotherapy Study Group, so as to permit thorough analysis of comparative clinical course and survival for experimental and control groups, randomly selected.

PITTSBURGH, UNIVERSITY OF (PH43-67-1186)

The main objective of this contract has been the structural determination of materials of interest to the program by X-ray crystallographic techniques. The project is limited to the study of materials whose structures are otherwise difficult to establish. This contract will terminate June 30, 1971.

POMONA COLLEGE (NIH-69-2112)

The project has two major objectives. First is the attempt to derive a structure-activity correlation for anticancer drugs. This will be done using currently available data and the statistical methods of linear regression analysis developed by Dr. C. Hansch of Pomona College. The second objective is the accumulation of fundamental data relating to hydrophobic bonding. These data will be used for further refinement of the structure-activity correlation as well as studies of the mechanisms of interaction of "minimolecules" (drugs) with macromolecules (enzymes).

PUBLIC HEALTH SERVICE DIVISION OF FEDERAL HEALTH PROGRAM SERVICES (TRANSFER OF FUNDS) (Baltimore, Maryland)

This transfer of funds permits service support by the Hospital of NCI-intramural cancer chemotherapy programs at the USPHS Hospital in Baltimore, Maryland. Programs of clinical pharmacology, clinical use of the newer drugs for the treatment of cancer, and developments in improved patient care are on-

PUBLIC HEALTH SERVICE DIVISION OF FEDERAL HEALTH PROGRAM SERVICES (TRANSFER OF FUNDS) (Baltimore, Maryland) (Continued)

going projects. Initial clinical trials of agents developed entirely within the NCI Chemotherapy Program and fundamental related laboratory research represent significant portions of the program.

RAWLEY FARMS (NIH-71-654)

This procurement contract provides 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

RAWLEY FARMS (NIH-71-669)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

RESEARCH TRIANGLE INSTITUTE (NIH-69-2019)

The purpose of this contract is to fractionate confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compounds. Plant material used for this work is obtained, for the most part, through the U.S. Department of Agriculture. The Contractor has many plants which are or have been in some stage of fractionation. Several active agents have been isolated and are currently being subjected to additional evaluation. One material, the soluble sodium derivative of camptothecin, is in clinical trial.

ROCHESTER, UNIVERSITY OF (NIH-69-39)

This contract is concerned with the biochemical pharmacology of antitumor agents in animals and man. The activities are directed specifically to improving the therapeutic effectiveness in man of existing drugs and to understanding the mode of action of drugs under development in the Chemotherapy Program area. The transport of drugs into human and animal cells and its relationship to the therapeutic activity of the agents is a major endeavor of the contract. The metabolic interactions and disposition of drugs are also studied with special emphasis on their relationship to cell growth and kinetics. The relationship of enzyme levels, DNA synthesis and drug sensitivity of tumors is investigated with the objective of developing improved therapeutic strategy. Investigation of the impact of clinical chemotherapy on solid tumors is undertaken by taking serial tumor biopsy specimens.

SCIENTIFIC LITERATURE CORPORATION (NIH-69-2114)

This contract provides for the preparation of Cancer Chemotherapy Abstracts (Volumes 9 and 10, for 1968 and 1969), a current awareness service, which supplies abstracts and annotations of the world's literature pertinent to

SCIENTIFIC LITERATURE CORPORATION (NIH-69-2114) (Continued)

cancer chemotherapy. Each volume is published in bimonthly issues followed by an annual cumulative index.

SCIENTIFIC LITERATURE CORPORATION (NIH-70-2267)

This contract provides for the preparation of Cancer Chemotherapy Abstracts (Volume 11, for 1970), a current awareness service, which supplies abstracts and annotations of the world's literature pertinent to cancer chemotherapy. The volume is published in bimonthly issues followed by an annual cumulative index.

SIMONSEN LABORATORIES, INC. (NIH-71-2101)

The Contractor maintains a genetic trust stock colony of 6 inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production propagation.

SIMONSEN LABORATORIES, INC. (NIH-71-639)

This procurement contract provides 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

SIMONSEN LABORATORIES, INC. (NIH-71-657)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

SOUTH SHORE LAB., INC. (PH43-64-932)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of 3-4 potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

SOUTHERN ANIMAL FARMS (NIH-71-640)

This procurement contract provides 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid

SOUTHERN ANIMAL FARMS (NIH-71-640) (Continued)

mice yearly for screening tests. Breeding animals originate from genetic centers and/or C57BL/6 and DBA/2 production colonies.

SOUTHERN ANIMAL FARMS (NIH-71-645)

This procurement contract is designed to furnish yearly 78,000 six-week-old CDF_1 (BALB/c♀ x DBA/2♂) hybrid mice for CCNSC contract studies. Breeding animals originate from genetic centers. The mice are offered chlorinated water in order to eliminate or control the incidence of pseudomonads.

SOUTHERN RESEARCH INSTITUTE (PH43-65-654)

The primary objective of this contract is to conduct studies on the development of quantitative methods for the estimation of antitumor drugs and their metabolites in biological media, and to apply these methods to in vivo and in vitro experimental systems for determining drug disposition. During the past contract year studies were conducted on the metabolism of cyclophosphamide (Cytoxan), arabinosyl-cytosine (ara-C), vincristine sulfate, Rifampicin, L-asparaginase, tetrahydrouridine, and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, using in vivo and in vitro systems. These studies are part of an integrated effort to provide more extensive and reliable basic quantitative data on the requirements of drug concentration (c) and time (t) to obtain complete tumor cell kill with minimal toxicity to the host.

SOUTHERN RESEARCH INSTITUTE (PH43-66-29)

The major objective of this contract is to develop basic knowledge useful in the design of new drugs or in the more effective use of known agents. To accomplish this aim studies are underway on: (a) sites of action and the metabolism of compounds with antitumor activity; (b) the biochemical basis for resistance to important antitumor agents; (c) the comparative biochemistry of tumors and various normal cells; and (d) study of the dynamics of cell growth and the effects of antitumor agents thereon.

SOUTHERN RESEARCH INSTITUTE (NIH-70-2083)

The purpose of this project is to develop toxicological information in primates and dogs which will allow for the safe, effective use of anticancer drugs in leukemia and in solid tumors. In addition, the project attempts to explore the many problems in definition of toxicities resulting from the use of combinations of antitumor drugs. During the past contract year, studies have primarily dealt with the nitrosourea derivatives, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), NSC 409962), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC 79037) and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU, NSC 95441).

SOUTHERN RESEARCH INSTITUTE (NIH-71-2021)

Southern Research Institute is one of the basic synthesis contractors for the CCNSC. The chemistry program is oriented to the biological and biochemical rationales developed in other programs, and is integrated with the screening,

SOUTHERN RESEARCH INSTITUTE (NIH-71-2021) (Continued)

therapeutic and biochemical activities at Southern Research Institute. The areas of investigation include: purine antimetabolites, including 9-substituted-9H-purines and ring analogs of purines; folic acid antagonists, directed toward the preparation of "simpler" derivatives and variants of the methotrexate and aminopterin structures; imidazole antimetabolites and analogs of imidazoles; nucleotides; N-nitrosourea analogs; derivatives of vitamin B_6; aryl and heterocyclic triazenes; metabolites of cytoxan; guanazole derivatives; and hydroxamic acid derivatives.

SOUTHERN RESEARCH INSTITUTE (NIH-71-2098)

This comprehensive research and development project is directly aimed at the mission of the NCI Chemotherapy Program. Objectives are (1) development of biological principles leading to discovery of more effective anti-cancer therapy, (2) development of improved assays for predicting clinical usefulness, and (3) the detailed evaluation of new drugs as potential clinical therapies. Objectives are realized through (a) basic studies of factors influencing growth of spontaneous and transplanted animal tumors, (b) studies of the influence of therapy on the dynamics of tumor cell proliferation, and (c) quantitation of drug activity relative to factors which influence it (e.g., treatment route and schedule). Studies of drug combinations and treatment of resistant tumors are conducted to uncover superior therapies leading to cures of animal tumors and in conjunction with studies of mechanisms of drug action and resistance. Mammalian cell culture studies are aimed at defining the influence of drug concentration and time at the target cell, and the cell cycle stage specificity of active drugs. Antimicrobial testing is aimed at discovery of suitable organisms for bioassay of effective drug levels in body fluids and to provide leads relative to mechanisms of drug action. Primary screening in vivo is conducted at a level appropriate for efficient maintenance of total project. A considerable portion of the effort in in vivo , cell culture, and antimicrobial screening is devoted to providing information relative to the basic and developmental studies under this contract and Southern Research contracts 66-29 (Biochemistry), 71-2021 (Organic Chemistry), and Pharmacology contracts (70-2083 and 65-654). This contract is a continuation of contract PH43-65-594.

STANFORD RESEARCH INSTITUTE (NIH-69-37)

The objective of this contract is the preparation of folic acid analogs, derivatives and their intermediates. The major emphasis is devoted to the preparation of selected materials determined to be key compounds by the structure-activity study and comprehensive review which was made in this area. The assignment of the compounds for preparation, whether new synthesis or resynthesis, is by CCNSC staff.

STANFORD RESEARCH INSTITUTE (NIH-71-2070)

Stanford Research Institute is one of the three major contractors devoted to

STANFORD RESEARCH INSTITUTE (NIH-71-2070) (Continued)

the synthesis of new chemical structures. The research program encompasses such areas as nucleosides, antimetabolites, antibiotics, alkaloids and other areas as may be requested by NCI.

The completion of the preparation of "homofolic acid" has opened the path for the synthesis of "homoaminopterin" and related compounds. Work is also in progress on other analogs of tetrahydrofolic acid, particularly compounds having high activity as inhibitors of dihydrofolic reductase and/or thymidylic synthetase. Exploitation of the leads in the Vinca alkaloid field is being pursued by synthesis of new indole alkaloids. Compounds related to the known indole alkaloids, ellipticine and olivacine, are also under investigation. Special attention is being directed to the study of the chemistry of the antibiotic, daunomycin, in order to develop agents of greater specificity at the molecular level. A modest effort is carried out in the polyhydroxyanthranquinone-type compounds. This structure occurs in daunomycin and many other antibiotics. Such materials often demonstrate a wide variety of antitumor activity in both animals and man. Associated with hydroxylated quinones are sugar moieties, which are also unique and merit investigation. These trideoxyamino sugars are also under investigation.

Over 40% of this contract is concerned with quality control studies on compounds prepared for preclinical toxicology and clinical trial. The Contractor evaluates bulk chemicals and formulation drugs as to their identity, assay and purity, to provide assurance of high quality of the necessary analytical data for the Food and Drug Administration.

This area of work provides elemental analysis, as well as the more sophisticated techniques such as chromatography (paper, thin layers, and gas), absorption spectroscopy (ultraviolet, visible, and infrared), nuclear magnetic resonance, and other physical-chemical procedures appropriate for the situation. In some cases methods of assay must be developed for new structures in order to maintain adequate surveillance on the quality of all drugs used in clinical trials.

STARKS ASSOCIATES, INC. (PH43-62-484)

This service preparative contract supplies the CCNSC with compounds required for drug evaluation studies, with 50% of the effort being devoted to the production of clinical materials. Preparations vary in quantity from 5 grams to multi-kilograms. Many of the compounds are potential folic acid antagonists and irreversible inhibitors.

STARKS ASSOCIATES, INC. (PH43-68-1275)

This contract is for the synthesis and/or resynthesis of nucleosides and their intermediates needed to complete structure-activity studies in this area. The major effort is on the resynthesis of designated nucleosides,

STARKS ASSOCIATES, INC. (PH43-68-1275) (Continued)

selected as key compounds for further study by the comprehensive review and structure-activity evaluation of nucleosides previously carried out. The assignment of compounds for preparation, whether new synthesis or resynthesis, originates with CCNSC.

TACONIC FARMS, INC. (NIH-71-651)

This procurement contract supplies 130,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

TEXAS INBRED MICE COMPANY (PH43-68-88)

This contract supplies a variety of inbred and hybrid rodents for tumor transplantation; for hybrid production (at Texas Inbred and other animal production contractors); and for compound evaluation studies. Animals are supplied from a colony of 25 strains of rodents. The Contractor also serves as a trust stock repository for the inbred strains of rodents.

TEXAS INBRED MICE COMPANY (NIH-71-658)

This procurement contract supplies 156,000 BDF_1 (C57BL/6♀ x DBA/2♂) hybrid mice yearly for screening tests. Breeding animals originate in genetic centers and/or C57BL/6 and DBA/2 production colonies.

TEXAS, UNIVERSITY OF (PH43-66-1156)

The objective of this contract is to conduct pharmacologic studies of antitumor agents in man, laboratory animals, and other predictive systems. The appropriate methodology is developed when necessary to conduct such studies. The compounds studied reflect the interests of the NCI Chemotherapy Program Area in developing antitumor drugs for clinical trial in man and exploring new dose levels and schedules in order to use these agents in the most effective and safe manner. Research on the contract during the past year mainly concerned various pharmacological studies on arabinosyl cytosine (ara-C), arabinosyl 6-mercaptopurine (ara-MP), β-2'-deoxythioguanosine (β-TGdR), 5-(3,3-dimethyltriazeno)imidazole-4-carboxamide (DIC), and 9,10-(bis-isothioureidomethyl)anthracene (BITU). The resulting data is integrated with pharmacologic data from other in-house and out-of-house cooperating pharmacology units of the NCI Chemotherapy Pharmacology Committee.

TEXAS, UNIVERSITY OF (PH43-67-1143)

This contract is concerned with the chronic toxicologic evaluation of antineoplastic drugs which are used for extended remission maintenance therapy and in the treatment of psoriasis, collagen diseases and for immunologic supression. Answers are being sought to the following questions: (1) will

TEXAS, UNIVERSITY OF (PH43-67-1143) (Continued)

the bone marrow continue to demonstrate reversibility of toxicity during a long course of serial insults; (2) what is the tolerance of the major organ systems to prolonged drug therapy and can function impairment be reversed by withdrawal of drug; and (3) what unusual toxicologic phenomenon might be encountered during the long-term administration of a specific agent?

THOMPSON, JOHN I., COMPANY (NIH-69-2158)

This contract is utilized to maintain and modify the accounting of the cost of drugs to the National Cancer Institute, and to keep a current record of such costs in total and by such variables as drug name, drug lot number, investigator, study group, NIH staff, clinical and non-clinical use of drugs, and other stratification. Data are derived from the records of the receipt of bulk chemicals or pharmaceuticals, and from clinical drug request and shipment forms.

TRW, INC. (PH43-63-622) (formerly HAZLETON LABORATORIES, INC.)

This contract provides *in vivo* screening of new test materials, both synthetics and natural products, and it provides subsequent testing of active materials.

The current testing level is approximately 22,000 tests per year. Murine L1210 Lymphoid Leukemia is the test system used routinely for all new materials. Testing against the intracerebrally implanted ependymoblastoma is being initiated, and testing against the intramuscularly implanted Walker Carcinosarcoma 256 is being phased out. Treatment schedule studies and research and development work are conducted as required by the Drug Evaluation Branch. Testing is conducted according to the CCNSC protocols for testing.

TRW, INC. (PH43-66-84) (formerly HAZLETON LABORATORIES, INC.)

The purposes of this contract are to develop various model tumor systems, including leukemia, lymphoma, and various solid tumors in primates; to study the potential carcinogenic effect of various compounds; and to maintain a primate breeding colony so that newborn and young primates may be used in chemotherapeutic, carcinogenic, and other pharmacologic and physiological studies. Owl monkeys are inoculated with *Herpesvirus saimiri* in an effort to develop a primate leukemia and lymphoma model. Thus far, four monkeys out of six inoculated have died of lymphoreticular neoplasm with leukemia. Certain antitumor agents, such as procarbazine, are being evaluated for carcinogenic activity. In addition, a variety of materials carcinogenic in rodents, as well as materials suspect of carcinogenicity in man are being tested in nonhuman primates for carcinogenicity. If model tumor systems are developed in primates, then these systems can be used to evaluate antitumor activity, pharmacology, and toxicology of various potential chemotherapeutic agents. Over 70 monkeys have had liver tumors induced by *N*-nitrosodiethylamine, and

TRW, INC. (PH43-66-84) (formerly HAZLETON LABORATORIES,INC.) (Continued)

1-nitrosopiperidine. Two monkeys have developed myelogenous leukemia after receiving procarbazine and four owl monkeys have developed lymphoreticular neoplasm with leukemia after inoculation with Herpesvirus. These monkeys are being utilized for chemotherapy, for t-RNA studies of normal and tumor tissue, for enzyme studies and to study potential diagnostic tests for animals and man with such tumors. Such information is of considerable importance to cancer research in general.

TRW, INC. (PH43-68-1286) (formerly HAZLETON LABORATORIES, INC.)

This contract provides for microbiological services in support of the NCI Chemotherapy clinical investigations at the Baltimore Cancer Research Center, and for special testing pertinent to this program. During the past year the services have also included microbiological surveillance and environmental studies in support of the Laminar Air Flow Room protocols of the National Cancer Institute, NIH, Bethesda, Maryland. Preliminary results of the studies are teletyped to the BCRC and NCI daily and used as indicated in research microbiological management of patients. The program employs a dataphone system for presumptive reporting and computer techniques for the retrieval of information as required by clinicians and research investigators.

TRW, INC. (NIH-69-2067) (formerly HAZLETON LABORATORIES, INC.)

The purpose of this contract has been to evaluate various aspects of the toxicity of potential cancer chemotherapeutic agents using mice, rats, dogs, and monkeys. This toxicologic evaluation is based on lethality, hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross pathology, light and electron microscope examination, and other parameters as indicated by the nature of past experience with various oncolytic agents. These studies have been carried out in close cooperation with NCI, and results are reported to the Laboratory of Toxicology, NCI. This contract is being terminated in Fiscal Year 1971.

TULANE UNIVERSITY (PH43-65-593)

This contract provides for the establishment of a foundation colony of caesarian-derived inbred rats (strains M520, OM/N, Fischer 344, Buffalo, W/L, ALB, ACI and other inbred strains as required). Its objectives are to improve the quality and maintain the genetic characteristics of the strains for program activities.

UPJOHN COMPANY (PH43-68-1023)

This contract has for its primary objective the development of potentially useful antitumor agents, from fungus and actinomycete fermentation beers, including isolation, purification, characterization, production, biochemistry, and pharmacology efforts.

UPJOHN COMPANY (PH43-68-1023) (Continued)

The Upjohn Company has played a leading role in the exploitation of cell culture methods, both for assays on fractionation samples and for primary screening. The present screen, aimed at the detection of new types of antitumor leads, consists of a search for drugs with specific antimetabolite activity, followed by the determination of activity in the L1210 and P388 tumor systems.

Major efforts in this contract are devoted to studies on the mechanism of action and physiological disposition of clinical drugs developed within the contract program, and phases of toxicological and pharmacological studies within the capability of the Contractor and requested by NCI.

UTAH, UNIVERSITY OF (PH43-65-1041)

This contract is for the synthesis of nucleoside analogs and derivatives, including nucleoside antibiotics, for screening as antitumor agents. The compounds selected for synthesis are based upon biological and biochemical rationale. The areas of investigation include: (1) analogs of antibiotic tubercidin and other nucleoside antibiotics, (2) imidazo[4,5-c]pyrimidine and imidazo[4,5-b]pyrazine nucleosides, (3) imidazole and benzimidazole nucleosides, (4) pyrazolo[3,4-d]pyrimidine and pyrazolo[4,3-d]pyrimidine nucleosides, and (5) nucleosides containing unsaturation in the carbohydrate moiety. This Contractor provides these difficult materials in quantities sufficient for in vivo antitumor evaluation by methods of synthesis developed by the Principal Investigator.

VETERANS ADMINISTRATION (D.C.) (TRANSFER OF FUNDS)

Funding is provided for a cooperative effort between the Veterans Administration and the National Cancer Institute for the clinical evaluation of potentially useful anticancer compounds. At the present time the program includes three elements: (1) the Veterans Administration Lung Cancer Cooperative Group, whose objective is to perform Phase II studies of promising new agents in metastatic lung cancer, (2) the VA Surgical Adjuvant Cooperative Group evaluates combined approaches of surgery plus drugs, and (3) the NCI-VA Medical Oncology Service at the Washington VA Hospital. This unit is responsible for experimental anti-tumor chemotherapy and related problems with emphasis on Phase I and early Phase II studies in bronchogenic carcinoma.

VIRGINIA, UNIVERSITY OF (NIH-71-2099)

This contract provides for the fractionation and isolation of active agents from plants with confirmed antitumor activity which stem from the program for screening of crude extracts and are assigned to contract chemists. During the current report period a number of crystalline materials were isolated and are now undergoing further study at various stages of evaluation.

VITRO LABORATORIES (NIH-69-58)

Data processing services are provided by this contract to the Chemotherapy Program and to the Endocrine Evaluation Branch of General Laboratories and Clinics (GL&C). The main areas of emphasis are: (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch, CCNSC (including computerized queries of the data files) and in the bioassay and endocrine-related tumor programs of the Endocrine Evaluation Branch (EEB); (2) completing the documentation of all CCNSC programs and contractor's procedures; (3) reprogramming the Update and Reporting subsystem of the EEB data processing system to be run at DCRT's computer facility and preparing specifications for the EEB Publications subsystem; and (4) providing programming assistance for the Clinical Master File System. Final copy for publication is also prepared by computer and conventional means.

WALTER REED GENERAL HOSPITAL (TRANSFER OF FUNDS)

This cooperative agreement and transfer of funds provides for the participation of Walter Reed General Hospital as a contributing member to the studies of the Acute Leukemia Cooperative Group B. Funds for professional and technical personnel, as well as consultant fees and miscellaneous costs, are provided.

WARF INSTITUTE, INC. (PH43-66-494)

This contract provides for the in vivo screening of new materials and the related assay and/or follow-up testing of active materials. Testing is conducted according to CCNSC protocols and the current test level is approximately 26,000 tests per annum. During the present reporting period all new compounds have been tested in lymphoid leukemia L1210 in the mouse host, with natural products also being tested in the P388 lymphocytic leukemia in the mouse. Testing in the Walker 256 intramuscular tumor in the rat host has been reduced to less than 5% of the total test level, since it is currently used only for confirmation testing of materials previously selected for development because of activity in Walker 256. Those materials without correlative activity in other systems will no longer be pursued. Schedule dependency studies on compounds of potential interest for clinical trial are conducted as required by program needs. Methodology and/or research and development is devoted to specific problems that arise in the routine screening of agents and is directed by the Project Officer.

WARF INSTITUTE, INC. (PH43-67-747)

This represents a service contract to prepare 50% aqueous-ethanol extracts of plant materials at the rate of approximately 2,500 extracts per year. A semi-quantitative test for alkaloids and tannins is run on plant materials of sufficient interest to be considered for fractionation. The majority of the Contractor's plants are obtained through a transfer of funds agreement between the NCI and the U. S. Department of Agriculture.

WEIZMANN INSTITUTE OF SCIENCE (PL-480 - Agreement No. NIH-06-025-1)

The objectives of this contract are: (1) to seek selectivity in cancer therapy through modification of the physical properties of anticancer agents by various oligomers and polymers, and (2) to produce polymers of amino acids which in themselves may have anticancer activity. The principal emphasis at present is on the interactions and relationships between these polymers and cell membranes.

WISCONSIN, UNIVERSITY OF (PH43-67-1126)

This contract, utilizing cell culture techniques, provides a rapid assay as an aid in the purification of potential antitumor agents from plant materials. The materials tested are the fractionation products prepared under Contract PH43-64-551 for the purpose of isolating the active agents from materials having established antitumor activity in the CCNSC *in vivo* screen. The principal methods used are an agar diffusion system and bioautography of paper chromatograms.

YALE UNIVERSITY SCHOOL OF MEDICINE (PH43-67-1193)

The objective of this contract is to provide *in vitro* screening systems to select promising anticancer agents of the antifolate type. The compounds are assayed for inhibition of dihydrofolate reductase from L1210 leukemia cells, from human chronic myelogenous leukemia and from normal red blood cells.

Some pharmacologic and metabolic work is done on selected agents of particular interest.

YALE UNIVERSITY SCHOOL OF MEDICINE (NIH-70-2089)

This service-type contract is primarily concerned with the preclinical toxicologic evaluation of three potentially useful antineoplastic agents in accordance with the protocols of the Laboratory of Toxicology. These studies are directed toward defining the qualitative and quantitative toxicity of these new drugs. The toxicity is characterized as to predictability, reversibility, relationship to cell cycle kinetics, and temporal relationship to termination of treatment. These characterizations are based on hematology, clinical chemistry, clinical observations, pharmacodynamic effects, gross and microscopic lesions and drug-induced death. Special investigations of drug-induced toxicity are conducted at the request of the Project Officer. All work is carried out in close cooperation with NCI and the results are reported to the Laboratory of Toxicology.

ANNUAL REPORT

CANCER THERAPY EVALUATION BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

The Cancer Therapy Evaluation Branch during the past year has been responsible for implementing and monitoring the clinical contract program designed to provide highly specific and immediate clinical trials of the anti-cancer drugs that have demonstrated high activity in animals in the preclinical phase of the chemotherapy program. The Branch has reviewed and evaluated the cancer therapy activities of the large clinical program performed in collaboration with the Veterans Administration and with other collaborative groups and individual physicians. The Branch has maintained a continuing liaison with various advisory and consultant groups established as working committees to study the effectiveness of specific types and methods of cancer therapy. The Branch has been responsible for the communication and filing of all information required by the Food and Drug Administration in connection with the drug development program of the National Cancer Institute.

The Chemotherapy Program Linear Array outlines the logical steps in the drug development program and enables the chemotherapy staff to analyse the entire spectrum of scientific activities in the light of more clearly defined goals. The CTEB has functioned as a monitor of the Decision Network aspect of this system, administratively handling the monthly Decision Network meetings.

During the past year seven new drugs have passed Decision Network 3 of the Chemotherapy Program Linear Array and have had IND applications prepared in the CTEB and filed with the Food and Drug Administration. They are outlined below:

1. 5-hydroxy-2-formyl-pyridine thiosemicarbazine (NSC-107392, 5-HP). This synthetic chemical was chosen for clinical trial on the basis of activity against leukemia L1210. Activity has also been demonstrated against Ca 755, Ehrlich ascites tumor and Lewis lung tumor. This agent appears to be cell cycle specific, showing best activity on a Q3H x 8 Q 4 day schedule (the optimal schedule for S-phase specific agents, such as Ara-C, hydroxyurea and guanazole). The molecular site of action of 5-HP appears to be specific hinderance of DNA synthesis through inhibition of the enzyme ribonucleotide reductase. In the dog and monkey, toxicology demonstrated renal tubular necrosis, dose related bone marrow suppression, nausea and vomiting, bizarre red blood cell morphology and hepatic impairment. Phase I studies are currently being performed at Sloan-Kettering Institute and at Yale University.

2. 5-Azacytidine. This pyrimidine analogue of cytidine was selected for clinical trial on the basis of its activity against mouse leukemia L1210. It was found to be most effective when given intraperitoneally by continuous treatment (maximum ILS: 290%). Evidence points to the fact that this agent

acts as an analogue of cytidine and is therefore incorporated into both RNA and DNA. By disrupting the processes of nucleic acid sequence into protein, the synthesis of protein is inhibited. In toxicologic studies the drug has been found to be a panmyelotoxin capable of causing widespread necrosis of lymphatic organs at higher doses. In contrast to the panmyelotoxicity, there was no detrimental effect on the epithelium of the gastrointestinal tract. Moderate single and repeated doses of the drug were damaging to the liver and significant elevations of BUN were consistently seen in animals receiving toxic doses of the drug. Phase I studies are in progress utilizing different schedules at Jefferson Medical College in Philadelphia, M.D. Anderson Hospital and Children's Hospital of Los Angeles.

3. I-propanol, 3.3'-iminodi-,dimethanesulfonate (ester), hydrochloride (NSC-102627). This synthetic chemical is a new sulfonic acid ester of aminoglycol. Its mechanism of action has not specifically been determined. It has been found to be active in a number of tumor lines, especailly against tumors naturally resistant to nitrogen mustard. In the L1210 tumor system it has shown significant activity and schedule dependency giving a maximum ILS of >322% on a day 1, 5, 9 schedule. Dose limiting toxicity in the dog is leukopenia with poor marrow recovery after repeated insults. Other toxic effects include hepatic toxicity in the form of altered liver function tests and gastrointestinal toxicity in the mouse. The drug has been used in Japan in the treatment of chronic myelogenous leukemia with good results. Phase I study is planned by the Central Oncology Group.

4. Adriamycin (NSC-123127). This drug is an antibiotic related to Daunorubicin (NSC-82151). Structurally, both share a pigmented aglycone in glycoside linkage with an aminosugar. Adriamycin differs only in the substitution of a hydroxyl group for a hydrogen in the acetyl radical in the hydroaromatic ring. Adriamycin demonstrated activity superior to Daunorubicin in L1210 mouse leukemia system, its most effective schedule being Q 3 hours on day 1 only. Also, the therapeutic index (lethal dose 10%/inhibiting dose 90%) of Adriamycin is more favorable than that of Daunorubicin (1.28 compared to 0.67). The drug appears to act by interfering with nucleic acid metabolism, especially inhibiting DNA synthesis and inducing bizarre chromosomal abnormalities. Initial Phase I and II studies by Italian investigators indicates activity in a spectrum of malignancy somewhat similar to that of Daunorubicin - leukemia, childhood solid tumors, lymphoma and some adult solid tumors. Currently, intensive investigations are ongoing in Acute Leukemia Group B, Children's Cancer Study Group A and the Southwestern Cancer Chemotherapy Cooperative Group.

5. Estradiol Mustard (NSC-112259). This compound is the 3, 17-diester of the steroidal hormone estradiol with the alkylating agent chlorophenacyl, and therefore is referred to as "estradiol mustard". Like Phenesterin, an ester of cholesterol with the same alkylating agent, it is effective against a number of animal tumors including Walker 256 carcinosarcoma: unlike Phenesterin, it has some hormonal effect. The toxicology of estradiol mustard reflects both the estrogenic and alkylating potential of the drug. Bitches showed endometrial hyperplasia and mammary symptoms: male dogs showed aspermatogenesis and squamous metaplasia of the prostate. In both sexes typical alkylating-agent myelosuppression was seen.

6. 1.2-di(3,5-dioxopiperazin-1-yl)-propane (ICRF-159, NSC-129943). This synthetic chemical was developed at the Imperial Cancer Research Fund Facilities in London. It was selected for clinical trials on the basis of anti-tumor activity in leukemia L1210 and the Lewis lung tumor. It has schedule dependency in the L1210 system (an intermittent schedule being superior to a daily one) and specifically inhibits DNA metabolism in the G_2-M phase of cell cycle growth. The principal toxicities encountered in animal studies were leukopenia and thrombopenia, with cytotoxic changes also noted in gastrointestinal, gonadal and lymphoid tissues. Initial clinical trials have been underway for over a year in Great Britain.

7. 1-2(2-chloroethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea (Me-CCNU, NSC-95541). This synthetic chemical was chosen for clinical trial based on its activity against leukemia L1210 and advanced Lewis lung tumor. Me-CCNU is an analogue of BCNU and CCNU which have received extensive clinical trial. The major reason for testing the new compound is its unique activity against advanced Lewis lung tumor which is far superior to that seen with either BCNU or CCNU. Phase I studies are planned by the Medicine Branch of the Chemotherapy Program.

It is traditional to divide the clinical trials of any new drug into three phases and this is true for neoplastic drugs as well. Over the years the Chemotherapy Program has developed its own concepts of these three phases with special emphasis on Phase I and II. These three phases of clinical trial from the point of view of the program are outlined below:

I. Clinical Pharmacology:

   A. Establish maximally tolerated dose at schedule(s) tested.

   B. Establish toxicity parameters and determine if toxicity predictable, reversible and/or treatable.

   C. Patients not required to have measurable disease - evidence of antitumor activity not essential.

II. Screening for Clinical Activity:

   A. Treat 10-20 evaluable patients with measurable disease in each of six "signal tumor types".

   B. Evaluate on the basis of objective response rate and characteristics of pharmacology, mechanism of action and cell cycle sensitivity.

III. Trial Under a Variety of Conditions to Permit Recommendations for General Usage:

   A. Controlled clinical trials.

   B. Combination studies.

Phase II has traditionally been a screen for antitumor activity. Inherent in any screen is a clear end point of response and a critical level of activity given this response which passes the compound on to the next step. The sensitivity of this activity level is determined by many factors including the activity of established agents in the field, the capacity to study drugs at the next level and the overall psychological expectation from the program involved and the related scientific community. This latter aspect of expectation is often what determines how critical individuals will be of a screen regardless of its intrinsic scientific validity.

The Chemotherapy Program has decided to use as its screen a panel of tumors which are labelled as signal tumor types. These are outlined below:

SIGNAL TUMOR TYPES

1. Adenocarcinoma of the Colon
2. Adenocarcinoma of the Breast
3. Bronchogenic Carcinoma
4. Acute Lymphatic Leukemia
5. Acute Myelocytic Leukemia
6. Non-Hodgkin's Poorly Differentiated Malignant Lymphoma

Adenocarcinoma of the breast and colon and bronchogenic carcinoma are used to screen against the slow growing tumors which are the most resistant to chemotherapeutic attack. The acute leukemias and lymphoma have been chosen to screen against the rapidly growing and more chemotherapeutically sensitive tumor types. It is felt that this kind of disease oriented Phase II trial is superior to the often used general Phase II trial in which a wide range of tumor types are treated, but only a few of these types in a number large enough to give meaningful results. It is not uncommon in the older literature to see a drug described as inactive based on a low percent of response in 50 or so patients, and to see that only 2 or 3 patients with colon cancer or breast cancer were treated.

Implicit in the usage of the six signal tumor types is a minimal definition of what constitutes an adequate clinical evaluation of a new antitumor agent. As a minimum it would be unfair to label a drug as "inactive" until it has been adequately evaluated in at least 10-15 evaluable patients in the six signal tumor types and found to have insufficient clinical activity in all of them. In line with this reasoning, the CTEB has instituted an intensive review of all cancer chemotherapeutic agents to see how many have been adequately evaluated by these standards and to provide an historical background for the data rapidly developing on the new investigational agents.

Below is an outline of the data available on the clinical antitumor activity on the commonly used commercially available agents in the six signal tumor types and malignant melanoma which is a tumor of high interest to the program. The data is given in percent objective response rate with the actual number of responses/total number of patients in parenthesis.

Clinical Activity for Commonly Used Antitumor Agents

Clinical Antitumor Activity*

Pooled Data in the Six Signal Tumor Types Plus Malignant Melanoma
Given in % with Number of Responses/Total Number of Patients in Parenthesis

	Acute Lymphatic Leukemia Induction	Acute Myelocytic Leukemia Induction	Lymphoma		Adeno-Carcinoma of Colon	Adeno-Carcinoma of Breast	Bronchogenic Carcinoma	Malignant Melanoma
			Hodgkin's Disease	LSA and RCS				
I. ALKYLATING AGENTS								
1. Cyclophosphamide	29 (101/347) CR = 13%	10 (8/76) CR = 2.6%	54 (245/452)	LSA 65 (173/276) RCS 56 (122/219)	16 (7/43)	32 (60/189)	33 (168/509)	26 (8/31)
2. Nitrogen Mustard	7 (2/29) "Acute Leukemia" All prior to 1950		63 (432/682)	LSA 49 (75/154) RCS 18 (3/17)	12 (6/50)	34 (33/98)	21 (81/391)	9 (3/34)
3. Chlorambucil	NE	16.7 (3/18)	61 (172/282)	LSA 67 (61/90) RCS 39 (18/46)	9 (5/55)	20 (11/54)	8 (2/23)	9 (2/22)
4. Melphalan (Phenylalanine Mustard)	0 (0/27)	NE	32 (22/70)		15 (12/82)	23 (20/86)	10.5 (4/38)	12 (8/66)
5. Busulfan (Myleran)	NE	3 (1/32)	25 (6/24)		10 (1/10)	0 (0/8)	32 (6/19)	10 (1/10)

Clinical Activity for Commonly Used Antitumor Agents

Clinical Antitumor Activity*

Pooled Data in the Six Signal Tumor Types Plus Malignant Melanoma
Given in % with Number of Responses/Total Number of Patients in Parenthesis

	Acute Lymphatic Leukemia Induction	Acute Myelocytic Leukemia Induction	Lymphoma		Adeno-Carcinoma of Colon	Adeno-Carcinoma of Breast	Bronchogenic Carcinoma	Malignant Melanoma
			Hodgkin's Disease	LSA and RCS				
II. ANTI-METABOLITES								
1. Methotrexate	40 (63/159)	14 (31/210)	30 (13/44)		17 (19/111)	34 (120/356)	33 (56/167)	9 (2/26)
2. 5-Fluorouracil	5 (1/21) All Old Studies		26 (6/23)		24 (305/1270)	28 (323/1152)	7.5 (12/158)	2.5 (1/42)
3. 6-Mercaptopurine	66 (393/596) CR = 38%	28 (144/521) CR = 11.5%	37 (13/35)		5 (4/75)	13 (6/45)	4 (4/95)	7 (2/30)
4. Cytosine Arabinoside	24 (41/168) CR = 8%	27 (101/502) CR = 20%	29 (12/42)		10 (10/103)	9 (6/65)	0 (0/60)	2 (1/51)
III. VINCA ALKALOIDS								
1. Vincristine	58 (191/327) CR = 44%	51 (21/41) CR = 25%	56 (74/132)	53 (109/204)	0 (0/26)	21 (47/226)	9 (4/43)	12 (6/53)
2. Vinblastine	9 (5/63)	26 (25/98) CR = 4%	65 (259/380)	27 (24/89)	7 (5/71)	20 (19/95)	11 (12/109)	15 (8/54)

Clinical Activity for Commonly Used Antitumor Agents

Clinical Antitumor Activity*

Pooled Data in the Six Signal Tumor Types Plus Malignant Melanoma
Given in % with Number of Responses/Total Number of Patients in Parenthesis

	Acute Lymphatic Leukemia Induction	Acute Myelocytic Leukemia Induction	Lymphoma		Adeno-Carcinoma of Colon	Adeno-Carcinoma of Breast	Bronchogenic Carcinoma	Malignant Melanoma
			Hodgkin's Disease	LSA and RCS				
IV. ANTIBIOTICS								
1. Actinomycin D	NE	NE	50 (9/18)		15 (7/48)	11 (5/45)	0 (0/16)	33 (19/58)
2. Mithramycin	NE	NE	40 (4/10)		16 (4/25)	16 (5/32)	0 (0/13)	15 (2/13)
3. Daunorubicin	27 (113/388) CR = 20%	37 (175/469) CR = 28%	18 (11/61)		0 (0/2)	0 (0/2)	0 (0/5)	0 (0/6)
V. MISCELLANEOUS AGENTS								
1. Hydroxyurea	12 (2/17)	16 (17/108) 3 CR	7 (1/14)		7 (4/59)	19 (4/21)	11 (4/36)	22 (47/213)
2. Procarbazine (Matulane)	0 (0/6)	20 (1/5)	70 (212/305) CR = 37%	40 (29/73)	20 (1/5)	5 (1/21)	15 (26/172)	18 (6/32)

* Clinical data summarized from files of Cancer Therapy Evaluation Branch, Chemotherapy, NCI
Figures for ALL and AML are for complete and partial remission
LSA = Lymphosarcoma
RCS = Reticulum cell sarcoma
NE = Not evaluated

It is clear from this data that most of these drugs have not been fully tested against all seven of these tumors. This data also reveals how tenuous a practice it is to list false positives for any particular preclinical screen for antitumor agents.

Currently the CTEB is responsible for monitoring the continuing trials of 46 non-hormonal investigational agents filed with FDA which include the seven new drugs discussed earlier. A listing of these in chronological order of IND filing with FDA with an outline of clinical results to date in the signal tumor types is given below. This list does not include the seven new drugs filed in the past year.

Name and NSC Number	Date IND Filed	Colon	Lung	Breast	Melanoma	ALL	AML	Lymphoma	Remarks
BCNU NSC-409962	6/63	13 (17/128)	9 (4/46)	35 (15/43)	20 (17/84)	NE	NE	HD 51 (71/151)	Activity also in brain tumors, multiple myeloma
Mitomycin C NSC-26980	6/63	20 (28/140)	21 (29/135)	37 (41/110)	18 (9/50)	NE	NE	47 (16/34)	NDA in preparation
Azotomycin NSC-56654	6/63	18 (25/143)	7 (4/56)	8 (3/37)	12 (6/50)	NE	NE	44 (4/9)	4/14 in pancreas 9/45 in sarcoma
Trimethyl-6-yl ammonium chloride NSC-51095	6/63	0 (0/4)	0 (0/7)	NE	NE	NE	NE	NE	Activity seen in transitional cell tumors of bladder
Trimethylcolchicinic acid (TMCA) NSC-36354	6/63	5 (2/37)	2 (1/42)	15 (2/13)	8 (4/49)	0 (0/6)	4 (2/47)	13 (7/54)	3/8 in sarcomatous disease
1-Aminocyclopentane-1-Carboxylic Acid (Cycloleucine) NSC-1026	6/63	12.5 (2/16)	NE	0 (0/4)	9 (1/11)	NE	NE	NE	Activity in leiomyosarcoma
5-IUDR NSC-39661	6/63	NE	NE	NE	NE	NE	NE	NE	All studies in combination; anti-viral activity
9-ethyl-6-mercaptopurine NSC-14575	6/63	NE	NE	NE	NE	Activity as remission maintainer NE for induction	50 (1/2)	NE	IND maintained for one patient
6-Azauridine NSC-32074	6/63	NE	NE	NE	NE	33 (13/39) All PR	0 (0/7)	17 (1/6)	Activity in choriocarcinoma
Methyl-GAG NSC-32946	6/63	0 (0/9)	12 (1/8)	16 (1/6)	NE	7 (1/15)	25 (75/297) CR = 15%	60 (12/20)	Activity reported in esophageal cancer

Name and NSC Number	Date IND Filed	Colon	Lung	Breast	Melanoma	ALL	AML	Lymphoma	Remarks
Streptonigrin NSC-45383	6/63	8 (5/60)	9 (6/68)	12 (4/24)	12 (1/6)	NE	NE	53 (16/30)	Slim therapeutic index
6-Mercaptopurine Riboside (6-MPR) NSC-4911	6/63	NE	NE	NE	NE	14 (19/130) All CR	34 (14/41) Only 1 CR	NE	
Hexamethylmelamine NSC-13875	7/63	15 (11/71)	21 (42/202)	28 (13/48)	6 (2/36)	NE	NE	85 (11/13)	Activity in ovarian tumors
Carbestrol NSC-19962	7/63	0 (0/8)	0 (0/17)	6 (1/12)	0 (0/2)	NE	NE	NE	Some activity in sarcomas
Porfiromycin NSC-56416	9/63	6 (3/49)	10 (4/40)	0 (0/9)	0 (0/8)	NE	NE	NE	Activity in squamous cell, cervix and head & neck
DL-ortho-Sarcolysin NSC-57199	11/63	0 (0/2)	20 (2/10)	25 (1/4)	0 (0/2)	NE	NE	NE	Activity in myeloma (9/15)
Azaserine NSC-742	3/64	NE	NE	NE	NE	NE	NE	NE	Almost all studies in combination. Currently reopened IV on new schedule
Tubercidin NSC-56408	5/64	0 (0/14)	0 (0/8)	33 (1/3)	0 (0/6)	NE	NE	NE	Activity in pancreatic cancer reported
6-Methylmercaptopurine riboside (6-MMPR)	8/64	NE	NE	NE	NE	0 (0/12)	0 (0/7)	NE	Most studies in combination with 6-MP
Phosphoramide mustard NSC-69945	10/64	50 (1/2)	0 (0/28)	NE	NE	NE	NE	NE	Active in vitro
5-BUDR NSC-38297	10/64	NE	NE	NE	NE	NE	NE	NE	All studies done with radiation

Name and NSC Number	Date IND Filed	Colon	Lung	Breast	Melanoma	ALL	AML	Lymphoma	Remarks
Pyran copolymer NSC-46015	3/65	NE	NE	NE	NE	NE	NE	NE	Only Phase I study performed
Ethidium chloride NSC-84423	9/65	NE	NE	NE	NE	NE	NE	NE	Only Phase I study performed
Pyrrolidine (Nafoxidine) NSC-70735	9/65	Discontinued 4/67 and reopened 7/69 for combination protocol in renal cell tumors							
8-Azaguanine NSC-749	9/65	NE	NE	NE	NE	NE	NE	NE	Used only in brain tumors
1-Acetyl-2-picolinoylhydrazine NSC-68626	11/65	4 (1/15)	0 (0/19)	10 (1/10)	0 (0/5)	0 (0/1)	0 (0/1)	8 (1/12)	
Dichloromethotrexate NSC-29630	3/66	NE	NE	NE	NE	14 (10/72) CR = 2	NE	32 (15/46) CR = 4	Not active in hepatoma
Imidazole carboxamide (DTIC) NSC-45388	5/66	11 (8/71)	10 (8/81)	10 (4/41)	20 (81/419)	NE	NE	40 (4/10)	Possible activity in leiomyosarcoma and brain tumors
F_3TDR NSC-75520	4/67	14 (2/14)	NE	20 (3/15)	NE	20 (2/10) 1 CR	NE	NE	
FUDR NSC-27640	6/67	23 (157/711)	13 (8/60)	34 (50/144)	10 (3/29)	25 (6/24) All PR	NE	NE	NDA for intra arterial use expected soon
Imidazole mustard NSC-82196	11/67	Oral formulation showed erratic absorption. Parenteral formulation currently in Phase I study.							

Name and NSC Number	Date IND Filed	Colon	Lung	Breast	Melanoma	ALL	AML	Lymphoma	Remarks
Etiocholanolone NSC-50908	12/67	Used for marrow stimulation studies Evaluation in solid tumors recently begun							
CCNU NSC-79037	2/68	15 (6/40)	11 (2/18)	NE	NE	NE	NE	Active in HD	Active in brain tumors
Dibromodulcitol NSC-104800	10/68	0 (0/15)	0 (0/41)	25 (8/32)	11 (3/27)	4 (2/51)	NE	28 (2/7)	
Camptothecin NSC-100880	12/68	14 (7/47)	NE	NE	NE	NE	NE	NE	
Acronycin NSC-403169	2/69	NE	NE	NE	NE	NE	NE	NE	Delay in starting Phase I study which is still ongoing
Emetine NSC-33669	7/69	NE	NE	NE	NE	NE	NE	NE	Variety of Phase II studies ongoing
Aniline mustard NSC-18429	7/69	NE	NE	NE	NE	NE	NE	NE	Activity seen in myeloma, prostate
Phenesterin NSC-104469	9/69	NE	NE	Minimal activity in 50 patients	NE	NE	NE	NE	
Poly I:C NSC-120919	12/69	NE	NE	NE	NE	NE	NE	NE	Phase I still ongoing
Bleomycin NSC-125066	4/70	NE	NE	NE	NE	NE	NE	~40%	Activity in squamous cell head & neck, and testicular
Beta deoxythio-guanosine NSC-71261	5/70	NE	NE	NE	NE	NE	NE	NE	

Data such as has been discussed does not even begin to take into account the question of whether an optimal or adequate dosage schedule was used. An optimal therapeutic schedule is a reflection of a variety of factors. In addition to the relative sensitivity of a given neoplastic cell as opposed to the normal cells of the body, there is the problem of the optimal body fluid concentration of drug with time. One cannot speak confidently about the relative sensitivity to drugs of various tumors in various hosts without having quantitative data on the blood concentrations of drug with time achieved in the comparative therapeutic trials and the degree of advancement and proliferative states of the respective tumor cell populations. While this can be obtained readily in experimental systems, such as in animals with leukemia L1210, it is rarely, if ever, obtained in human clinical trials.

An excellent example of the problems involved in evaluating a compound as active or inactive without considering dosage schedule is Methotrexate. This drug is one of the oldest in the relatively short history of modern cancer chemotherapy. In leukemia L1210, schedule dependency was shown for MTX by Goldin and Venditti in two situations: against "early" L1210 (one or two days after inoculation) and against advanced L1210 leukemia after other agents had been used to markedly reduce the leukemia cell load. On the other hand, they found that daily administration of the drug resulted in optimal therapeutic effect against "advanced" disease (in which treatment was not started until 7 or 8 days after inoculation, at a time when the mice had an enormous leukemia cell load). It has since been amply demonstrated that intermittent (twice weekly) MTX is superior to daily MTX for remission maintenance but not for induction of childhood acute lymphatic leukemia.

Methotrexate has been used clinically on a variety of schedules and it is of interest to look at the pooled reported results in the literature for the signal tumor types and two other tumors broken down by dosage schedule. This is shown below:

Comparison of Objective Response Rates
to MTX in 8 Tumors
Correlated With Schedule Used

Tumor Type	Single Dose Daily in %	Divided Dose Daily or Cont. Inf. in %	High Single Dose Intermittent in %	Twice Wkly or Wkly in %	Loading Course Monthly
ALL	35 (51/144) CR=27	-	80 (12/15)	31*	-
AML	13 (12/92)	25 (10/40) CR=17.5	-	9 (7/78)	-
Lymphoma	30 (13/44)	-	-	-	-
Breast	41.5 (44/106)	37 (38/102)	-	40 (15/38)	21 (23/110)
Colon	33 (2/6)	23 (9/39)	25 (3/12)	-	9.2 (5/54)
Lung	-	27 (5/18)	25 (5/20)	39 (39/100)	24 (7/29)
Malignant Melanoma	0 (0/3)	14 (2/14)	-	-	0 (0/9)
Head and Neck	-	33 (13/39)	38 (11/29)	55.8 (43/77)	39 (34/87)

* Reported without numbers given - a randomized comparison with daily MTX which gave 28%.

The data above reveal that Methotrexate has not received an adequate trial in any of these 8 major clinical tumor types on each of the five most commonly used dosage schedules for the drug. Clearly it would be a mistake to call MTX "inactive" in breast cancer, colon cancer or lung cancer based on the available data. This data points out the hazardousness of labelling drugs as false positive for a given screening system as is often done for leukemia L1210 and activity in solid tumors.

It is obvious from all of this discussion that greater emphasis will have to be placed on defining what constitutes an adequate trial for a given drug in a given human tumor system. Trials will have to be individualized for each kind of human tumor, taking cognizance of the pharmacology and schedule dependency of the drug and its potential interaction with what is known about the cell kinetics of the tumor.

In line with the disease oriented approach to new drug testing, a clinical contract has been undertaken with the Mayo Clinic for the evaluation of new drugs in gastrointestinal cancer, especially adenocarcinoma of the colon. The Chief, CTEB has functioned as Project Officer for this contract. Since the onset of this contract (18 months ago), a total of 187 patients have been treated on 7 protocols as outlined below:

1. Dimethyl triazeno imidazole carboxamide (NSC-45388): 30 patients with advanced gastrointestinal cancer were given 5 days courses in daily doses ranging from 200-600 mg/m^2. The most frequent toxic reactions were nausea and vomiting, facial paresthesias and bone marrow depression affecting all cell lines. Hematologic toxicity was unpredictable in occurrance and frequently severe at the higher dosages studied. Only 3 patients had objective evidence of antineoplastic effect. This was incomplete, transient, and of no significant clinical value to the patients.

2. Streptozotocin: 38 patients with advanced gastrointestinal cancer were treated at dosages of either 500 mg/m^2/day x 5 or 1000 mg/m^2/week x 4. Side effects included nausea, vomiting, and transient and reversible renal, hepatic and pancreatic beta cell toxicity. Two of 32 patients with adenocarcinoma had brief and incomplete objective responses. Of four evaluable patients with the malignant carcinoid syndrome, improvement in the syndrome was observed in three and objective improvement in two. These responses were also of short duration. A single patient with a non-functioning islet cell carcinoma of the pancreas had a striking symptomatic and objective response maintained for ten months.

3. Emetine: This study continues with 17 patients treated and evaluable to date. 12 patients were treated on a schedule of 1 mg/kg/day x 10 with one transient response being seen. Study will now shift to an intermittent schedule which has been shown to be superior in experimental systems.

4. Camptothecin: 55 patients have been treated on this highly important study which is still in progress. This expensive natural product was shown in Phase I study to have indications of activity in advanced gastrointestinal cancer. 32 evaluable patients have been treated at the Mayo Clinic on a single dose schedule of from 90-180 mg/m^2 repeated every 3 weeks. Three partial

responses of >50% have been observed and an additional 3 incomplete responses (25-50%) have also been reported. Twelve evaluable patients have been treated on a daily x 5 schedule with no objective responses being seen. The toxicity observed has included nausea, vomiting, diarrhea, stomatitis, cystitis, alopecia and marrow suppression.

5. CCNU: 41 patients have been treated on a schedule of 130 mg/m^2 PO single dose every six weeks. Six objective responses have been observed with a median duration of 10 + weeks with 3 patients still in remission. The major toxicity has been leukopenia, thrombocytopenia and gastrointestinal toxicity predominantly manifesting as nausea and vomiting.

6. Studies just beginning include a combination approach involving a full dose of CCNU (130 mg/m^2) to be followed in six weeks by a full course of 5-Fluorouracil. The other new studies includes an evaluation of Bleomycin alone and to be followed by CCNU. In addition Bleomycin plus radiation will be evaluated.

During the course of the year new or revised clinical brochures were written on the following drugs:

1. 1-Aminocyclopentane carboxylic acid (Cycloleucine), NSC-1026
2. Dimethyl triazeno imidazole carboxamide, NSC-45388
3. CCNU, NSC-79037
4. Bis (3-mesyloxypropyl) amine hydrochloride, NSC-102627
5. 5-Azacytidine, NSC-102816
6. 5-hydroxy-2-formyl-pyridine thiosemicarbazone, NSC-107392
7. Adriamycin, NSC-123127
8. Bleomycin, NSC-125066
9. BCNU, NSC-409962

During the past year the CTEB has functioned as the operations office for the Brain Tumor Study Group. The Chief, CTEB has functioned as Executive Secretary of the group and as the Project Officer on the clinical contracts which support the group. The group is currently evaluating BCNU in the treatment with malignant glioma. Patients are all given maximal surgery and then randomized as follows:

1. Best supportive care
2. BCNU
3. Radiation
4. BCNU and radiation

The end point will be survival, but the group is also attempting to define recurrent disease in order to see if it correlates with survival and can be used as an end point for evaluation.

Patient accrual on the study has been excellent with approximately 150 patients having been entered.

Presentations

1. Carter, S.K., Daunorubicin and Cardiac Toxicity presented at the Baltimore Cancer Research Center, April 30, 1970.

2. Carter, S.K., The Chemotherapy of Malignant Melanoma presented at the Tulane University Symposium on Malignant Melanoma, New Orleans, May 30, 1970.

3. Carter, S.K., Some Thoughts on the Therapy of Childhood Acute Leukemia presented at the Chemotherapy Program Review, Washingtonian Hotel, Gaithersburg, Maryland, July, 1970.

4. Carter, S.K., New Agents Developed by the Chemotherapy Program presented at the European Association for Cancer Research, Brussels, September 15, 1970.

5. Carter, S.K., The Evaluation of New Agents and the Chemotherapy of Breast Cancer presented at the Institute Jules Bordet, Brussels, September 17, 1970.

6. Carter, S.K., New Agents Developed by the Chemotherapy Program of the National Cancer Institute presented at the Institute Gustav Roussy, Villejuif, France, September 20, 1970.

7. Carter, S.K., New Investigational Agents presented at Medical Oncology Course, Somerset Hospital, Somerset, New Jersey, December 3, 1970.

8. Carter, S.K., Some Thoughts on Phase II Trials presented at the Symposium on the Statistical Aspects of Experimental Design, San Juan, Puerto Rico, December 9, 1970.

9. Carter, S.K., Clinical Trials and Combination Chemotherapy presented at Chemotherapy Contracts Meeting, Annapolis Hilton, December 18, 1970.

10. Carter, S.K., Chemotherapy of Lung Cancer presented at Symposium on Lung Tumors at Loma Linda University, Loma Linda, California, January 13, 1971.

11. Carter, S.K., New Investigational Drugs presented in Current Concepts in Medical Oncology sponsored by Mount Zion Hospital and Medical Center, San Francisco, January 15, 1971.

12. Carter, S.K., New Thoughts on Melanoma Therapy presented in Current Concepts in Medical Oncology sponsored by Mount Zion Hospital and Medical Center, San Francisco, January 15, 1971.

Publications

1. Livingston, R.B. and Carter, S.K.; Daunomycin. A Review. Chemotherapy Fact Sheet, March, 1970.

2. Carter, S.K.; 1-Aminocyclopentane Carboxylic Acid (NSC-1026) - A Review. Chemotherapy Fact Sheet, July, 1970.

3. Carter, S.K.; Cancer Chemotherapy in Western Europe as Observed at a Post-Graduate Course in Milan. Current Developments in the International Health Sciences prepared by the John E. Fogerty International Center for Advanced Study in the Health Sciences, September, 1970.

4. Livingston, R.B. and Carter, S.K.; Single Agents in Cancer Chemotherapy, 405 pages, Plenum Press, New York City, December 1970.

5. Carter, S.K., Editor; Proceedings of the Chemotherapy Conference on the Chemotherapy of Solid Tumors: An Appraisal of 5-Fluorouracil and BCNU, January 29-30, 1970.

6. Carter, S.K. and Livingston, R.B.; Hexamethylmelamine (NSC-13875) - A Review. Chemotherapy Fact Sheet, February, 1971.

7. Carter, S.K., Broder, L. and Friedman, M.; Streptozotocin and Metastatic Insulinoma - An Editorial. Annals of Internal Medicine 74: 445-447, 1971.

8. Livingston, R.B., Venditti, J.M., Cooney, D.A. and Carter, S.K.; Glutamine Antagonists in Chemotherapy. Advances in Pharmacology and Chemotherapy, Vol 8:57-120, 1970.

SUMMARY

OFFICE OF THE CHIEF

BALTIMORE CANCER RESEARCH CENTER

This past year's efforts have resulted in several clinical and laboratory observations which may alter importantly the modern management of patients with cancer treated by drugs. In regard to direct patient care, considerable improvements in infection control and prevention have been afforded through the pioneer work in surveillance microbiological cultural technique, defining more precisely the patient at risk from infection and criteria for appropriate early institution of appropriate antibiotic therapy. These programs complemented with an increasing use of the protected patient environment (laminar flow) have permitted a more confident and effective use of drugs to induce remission in leukemia and various solid tumors. A unique contract microbiology program and data retrieval system, perhaps the only such research quality level program in the country, is being broadened to facilitate patient care services to other selected clinical areas of the NCI Chemotherapy Program. A similar approach is being considered in the area of viral diagnostics.

Clinical pharmacologic studies with Daunomycin have again demonstrated the degree of collaboration between the clinic and the laboratory which evaluates drug metabolism, resistance and mechanism of action pari passu with therapeutic programs. Of interest is that efficacy of daunomycin appears related to both drug uptake and its intracellular metabolism to a metabolite, daunorubicinol, which has both toxicity and in vitro tumor cell kill. New studies with guanazole are underway.

The program of chemotherapy of CNS malignancy has been predominantly concerned to date with dose scheduling of the nitrosoureas. Programs for CNS leukemia control should be enhanced by work with the cytocentrifuge. This device, by permitting cell concentration of the cerebrospinal fluid, has been demonstrated to detect meningeal leukemia before other diagnostic tests are positive and should permit more effective therapy of this increasingly common complication. The laboratory efforts of animal toxicity of intracerebral chemotherapy can be anticipated to enhance markedly such therapeutic approaches.

Earlier work on exploratory laparotomy in malignant lymphoma has resulted, at least in part, for this diagnostic procedure to have passed into general accepted use throughout the country.

An interesting observation which requires clarification is that the liver and spleen of many patients with Hodgkin's disease contain non-diagnostic granuloma, cellular infiltrates and, rarely, necrosis. Classic staining techniques have failed to elucidate the nature of these lesions and they may offer a clue to disease etiology and prognosis.

An area which should be followed carefully is future developments in polyamine metabolism of clinical cancer. The amines putriscene, spermine and spermidine appear in high concentrations in the urine of patients with cancer but, to date, not in normals or patients with non-malignant diseases. A rough correlation, currently being defined more precisely, between the presence and extent of cancer and polyamine urinary level is evident. Work with specific polynucleases and similar enzymic approaches whould result in analytic techniques characterizing the products of malignant cells as distinct from normal tissue. This area of tumor cell assay systems represents a major effort of the program.

The prospects of an effective immunologic attack on certain cancers were improved by the development of an in vivo system for generating specific anti-tumor antibodies utilizing an interesting modification of the human anti-Rh desensitization technique. Work on this area could result in a major clinical breakthrough.

The clinical area in general has remained vigorous, in spite of the unsettling political events related to potential closure of the PHS hospitals. For this fiscal year, over 350 patients will have been treated utilizing 35 beds and over 1300 clinic visits. The disease spectrum of leukemias, lymphomas, melanomas, brain tumors and the more common cancers characterizes the program. Well over two dozen experimental and conventional chemotherapeutic agents have been employed. Their use has been facilitated and monitored through the continued development of the patient care pharmacy service. This activity has continued to demonstrate its relevance to modern research programs in drug therapy. A heartening feature of the past year has been the broad demonstration of political, community, patient and program support of the cancer project in Baltimore. The mood remains positive and expansionistic and it is hoped that future growth of the program will justify this attitude.

Serial No. NCI 6217
1. BCRC
2. Office of Chief
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of the Value of a Laminar Air Flow Isolation Unit and Oral, Non-absorbable Antibiotics in the Therapy of Patients with Acute Myelocytic Leukemia

Previous Serial Number: Same

Principal Investigator: Jerome B. Block

Other Investigators: Viola Mae Young, Stephen C. Schimpff, William Greene, Lois Jepsen, and Clarence Fortner

Cooperating Units: Litton Industries; Biomedical Engineering and Instrumentation Branch, NIH; Hazleton Laboratories, Virginia

Man Years

Total:	4
Professional:	3
Other:	1

Project Description:

Objectives:

The major limiting factor today in the therapy of patients with acute myelocytic leukemia is infection. The ultimate objective of the program is to reduce the incidence of infectious death occurring before adequate cancer chemotherapy could be given. However, to arrive at the final conclusion will require not only an analysis of infections but also an analysis of 1) remission incidence, 2) costs, both economic and emotional, 3) microbiology and its association with infections observed, 4) problems in function and management of the system, and 5) problems in design of a system with potential value on a "regular" medical ward.

Major Findings:

A. Infections - Nineteen patient studies have been performed since July 1970. Three patients have been placed in the LAF with two given and one not given oral, non-absorbable antibiotics. Nine patients have been treated with ward care alone and five patients have been treated seven times on the ward with the oral antibiotic regimen. A total of 42 infections, 10 associated with bacteremia, have been observed over the 699 days of patient studies. Too few patients have been studied to establish if any of these

means of therapy is more efficacious; however, there is a tendency to believe that those on the oral antibiotics are having fewer significant infections and a higher incidence of remissions.

B. Program Evaluation - 1) Physical and Personnel - It has clearly been determined that one laminar air flow room can be integrated into a medical ward setting but that to do so requires additional supporting personnel and/or markedly increased effort by the present staff. Personnel refers to all categories - physicians, nurses, pharmacists, housekeepers, maintenance, etc. We are in the process of evaluating the economic costs but, since this is a prototype program, it is expected that our costs for this year will be higher than similar future programs. With the recent addition of a second unit, the costs in money and personnel have again risen significantly but it would appear that operation of two units does not require twice the expense of one unit. 2) Patient - Only patients with average or above intelligence and high motivation appear suitable to therapy in the LAF. This may create a bias in evaluation.

C. Program Planning - 1) Present Units - Weekly meetings have been held to review a) the status of each patient in the program, b) the problems of physicians, nurses, etc. in working with the project, and c) in maintaining a continuous evaluation of the overall program. In April of 1971, a complete review of the entire program was conducted. 2) Future Units - Considerable time has been spent planning a medical program and the physical layout for an eight bed ward. This was to have been originally completed by January 1, 1971 but innumerable delays, all arising outside of the BCRC, now leave us with no predicted date of construction completion.

D. Microbiology -- See Annual Report No. 6209.

Proposed Course:

1) Continue studies with present laminar flow units.
2) Continue evaluation of program and units.
3) Continue to plan for proposed 8 bed ward.
4) Continue microbiologic studies, including viral isolation.

Significance to Cancer Research:

Patients with acute myelocytic leukemia frequently die before they have had an adequate trial of chemotherapy; death is usually due to infection. If a means can be found to reduce these infections, perhaps more patients will reach complete remission.

Honors and Awards: None

Publications: None

SUMMARY REPORT

Clinical Branch, Baltimore Cancer Research Center, NCI
July 1, 1970 through June 30, 1971

Several new projects have been brought towards completion this year. A pilot randomized study of chemotherapy and x-irradiation employed sequentially for the treatment of early (Stage I-II) Hodgkin's disease has shown that local radiotherapy followed by multiple courses of combination drug therapy (MOPP) is more effective than the reversal sequence in preventing recurrence of the disease in non-irradiated areas. New approaches toward improving the excellent response of more advanced (Stage III B and IV B) Hodgkin's disease with new drug combinations are being explored. Exploratory laparotomy in early Hodgkin's disease has continued and confirmed the early impression of the inadequacy of clinical and radiological criteria in assessing disease below the diaphragm. While the question of diagnostic laparotomy would now seem to be no longer of research investigative interest, the program will continue to complement planned Gallium 67 scanning studies in lymphoma.

Clinical studies with Daunomycin have been extended and show that large (180 mg/m^2) intermittent doses of the agent represent an apparently effective mode of remission induction for acute, adult, non-lymphocytic leukemia. These studies have been complemented by concomitant evaluation of drug metabolism in man. Over the past few years, both single large dose therapy and daily therapy with the agent have been sequentially evaluated. Future studies in this disease will be concerned with new agents for induction such as guanazole and camptothecin and probing studies for better maintenance of the remission state.

Continued evaluation of the nitrosoureas for primary and metastatic malignancy of the central nervous system has confirmed the early clinical interest in these patients. With regard to central nervous system leukemia, the use of the cytocentrifuge has given the physician an improved diagnostic technique for early detection of minimal or persistent disease.

A considerable constellation of effort has been centered around the problem of infection in patients, an effort justified when one appreciates the causes of death and morbidity in our patient group. Carbenecillin and gentamicin have been shown, through these studies, to change and control the spectrum of gram negative disease. A unique system of microbiological surveillance of patients at risk has identified almost all patients susceptible to infection prior to clinical disease. Experience

with laminar flow has increased with two units in operation. The data increasing point to the efficacy of such an approach to infectious sequelae of leukemia.

The program of blood component therapy is in routine operation. Of interest is the demonstration of platelet contamination and bacterial growth in units stored at room temperatures.

Serial No.: NCI-6027
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Microbiological Services and Research Studies for Cancer Patients on a Chemotherapy Program

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Gerald Vermeulen, M.D., Dolores M. Kenton, B.S., Norman R. Friedman, B.S., William H. Shackelford, B.S., Arthur S. Levine, M.D., Edward S. Henderson, M.D., Janet Hauser, B.S.

Cooperating Units: Leukemia Service, National Cancer Institute, N.I.H., Bethesda, Md.; Hazelton Laboratories, Reston, Virginia.

Man Years (July 1, 1970 - June 30, 1971):

Total: 2-1/2
Professional: 1
Other: 1-1/2

Project Description:

Objectives:

1. To supply microbiological support of the quality and versatility necessary for cancer research programs and to provide information that may lead to prophylactic as well as therapeutic measures against infection in cancer patients.

2. To carry out studies, environmental as well as ecological investigations of the patient flora, in support of individuals with cancer who will be placed in the microbiologically controlled area of laminar air flow rooms.

Methods Employed:

The methods are the same as those previously described except that they have been expanded to include the samples collected at Leukemia Service, N.C.I., N.I.H. as well as those at B.C.R.C.

Major Findings:

The acceptance accorded the data generated by this study by clinical and

research staff is evidenced by its expansion to include surveillance cultures from the Laminar Air Flow Studies at the Leukemia Service, N.C.I., N.I.H. The data are used for both patient management and as a basis for various other projects as mentioned in the previous report. The information has been recorded on magnetic tape and is being retrieved for evaluation (See Project Nos.: 6264, 6266, 6254, 6257,) etc.

Significance to Bio-medical Research:

This program has proven the acceptability of an outside contract facility for support of the microbiologic needs of a clinical and research staff. It is providing the basis for better understanding of infection in cancer patients and for the control of many such infections.

Proposed Course:

To continue the program which has proven satisfactory and to include as a part of its objective the surveillance of cancer patients in the environment of a laminar air flow room.

Honors and Awards: None

Presentations: None

Publications: None

Serial NO.: NCI-6029
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Environmental Control of Bacteria Potentially Pathogenic for Cancer Research Patients

Previous Serial No.: Same (Also related to Serial No. 6217, project on Laminar Air Flow Rooms)

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Dolores M. Kenton, B.S., Norman R. Friedman, B.S., Barry L. Hainer

Cooperating Unit: National Communicable Disease Center, Atlanta, Georgia.

Man Years: (July 1, 1970 - June 30, 1971):

Total: 1-1/4
Professional: 3/4
Other: 1/2

Project Description:

Objectives:

Cancer patients who are undergoing chemotherapy or who have been placed on immunosuppressive drugs are highly susceptible to bacterial infection, even with bacteria which are considered saprophytic. The chief objective was to free the hospital environment from bacteria potentially pathogenic for cancer patients. The second objective was to provide a microbiologically stable environment so that the study of the endogenous flora can be carried out in a controlled situation.

Methods Employed:

Routine medical and environmental microbiological testing techniques. All Pseudomonas are serotyped (Fisher method) and all Staphylococcus aureus are phaged typed.

Major Findings:

Surveillance culturing of floors revealed that more frequent wet mopping was necessary to prevent build-up of large numbers of Bacillusspores in the environment.

Routine culturing of the Laminar Air Flow Rooms including floors, bedside tables, beds, telephones, sinks, showers, toilets, etc., showed but few microorganisms except for areas of immediate patient contact and the floors. The wearing of booties was instituted to help prevent floor contamination. Microorganisms recovered reflected patient flora with few exceptions excluding the floor at the entrance to the room. The cultures serve as a warning of microbial contamination of sink and shower drains, water supplies, malfunctioning filters, etc. Such surveillance is essential to the function of the LAF room.

Significance to Bio-medical Research:

To prevent the spread of bacteria within the hospital environment which may prove pathogenic and to provide a basis for using and evaluating the protective isolation afforded by Laminar Air Flow Rooms.

Proposed Course:

1. To continue monitoring cleanliness of the environment.

2. To check personnel, particularly those in contact with TICU and Laminar Air Flow Room (LAF) patients on a regular basis for the presence of bacterial pathogens.

3. To provide intensive environmental studies for the LAF areas including also food and supplies.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6031
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: A Proposed New Genus of the Family Enterobacteriaceae

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Dolores M. Kenton, B.S., Barbara J. Hobbs, Marcia R. Moody, M.S.

Cooperating Units: Clinical Pathology Department, Clinical Center, NIH, Bethesda, Md.

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/16
Professional: 1/16
Other:

Project Description:

Objectives:

A group of microorganisms was collected from patient samples for a period of over two years which did not give reactions compatible with the description of any known group of microorganisms extant. This study delineates these bacteria according to standard biochemical and serological differentiation procedures and will establish reported differences in their sensitivity patterns from other Enterobacteriaceae.

Methods Employed:

Standard methods were used.

Major Findings:

These microorganisms which were found not to conform to descriptions of any other genus of the family Enterobacteriaceae have been accepted by the American Type Culture Collection and submitted to the Type Culture Collection in England as a new genus with two species: Levinea malonatica and Levinea amalonatica. Strains have been exchanged with a number of individuals here and abroad who have expressed interest in them. Problems were encountered in the g-c ratios and strains were re-submitted for evaluation. Final acceptance of these microorganisms as a new genus, or as a new species

of Citrobacter, is under consideration by the International Nomenclature Committee. In contrast to Enterobacter and Citrobacter, which these microorganisms most closely resemble, the Levinea tend to be susceptible to the antibiotic cephalothin. Thus far no serological cross reactions between Levinea and these two genera have been demonstrated.

Significance to Bio-medical Research:

These microorganisms are among the opportunistic microorganisms that invade immunosuppressed patients. It is important to establish their identity as they often differ significantly in antibiotic sensitivity from the genera which they most closely resemble.

Proposed Course:

With the exception of collaborating with other microbiologists exchanging strains and awaiting the outcome of the decision of the Nomenclature Committee, no further work on this project is proposed.

Honors and Awards: None

Presentations:

Levinea, a New Genus of the Family Enterobacteriaceae Recovered from Patient Samples, presented at the Tenth International Congress for Microbiology in Mexico City.

Publications:

Due to delays occasioned by change of editor the publication slated for last year entitled: "Levinea, a New Genus of the Family Enterobacteraceae" in the International Journal of Systematic Bacteriology is due to appear in the first issue of 1971 under the new editor.

Serial No.: NCI-6035
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Incidence of Antibodies against Heterogenetic Enterobacterial Antigen in Typhoid Vaccinated Subjects

Previous Serial Number: Same

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D.

Cooperating Units: USPHS Outpatient Clinic, Washington, D.C.

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/20
Professional: 1/20
Other:

Project Description:

Objectives:

A heterogenetic antigen common to many members of the family Enterobacteriaceae has been reported. It has also been suggested that antibody against this antigen may play a protective role against infection by members of this family of bacteria. This study was undertaken to determine a possible heterogenetic antibody response to vaccination with Salmonella A,B and D vaccine, to compare it with antibody response to the vaccine itself and to compare it to any possible amnestic response.

Methods Employed:

High titered heterogenetic antisera were prepared by use of Escherichia coli serotype 014 which contains large amounts of heterogenetic antigen. Hemagglutination tests were used to detect heterogenetic antibody and both hemagglutination and agglutination tests to detect antibody response to the vaccine.

Major Findings:

Other than the findings reported the previous year an attempt was made to determine the presence of heterogenetic antigen in one lot of Salmonella vaccine with negative results.

Significance to Bio-medical Research:

To clearly understand the immune process in Salmonella vaccination, it is necessary to determine the heterogenetic antibody response as well as to compare it with the antibody response to the Salmonella somatic antigens used. It is also necessary to know if heterogenetic antibody is present in the vaccine used.

Proposed Course:

Extraction of other lots of Salmonella vaccine to determine presence or absence of heterogenetic antigen will be performed if there is sufficient time of technical personnel to carry out the work.

Honors and Awards: None

Presentations: None

Publications: None

Serial No. : NCI-6036
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Broad Spectrum Bacterial Antibody Evaluation of Cancer Patient Sera

Previous Serial No.: None

Principal Investigator: Marcia R. Moody, M.S.

Other Investigator: Viola Mae Young, Ph.D.

Cooperating Units: None

Man Years (July 1, 1970 - June 30, 1971)

Total: 1/12
Professional: 1/12
Other: 0

Project Description:

Objectives:

It has been reported that serum antibody response to various antigenic stimuli is altered in cancer patients. However, variation occurs from one antigen to another. The broad spectrum antibody screen is intended to determine "normal" antibody levels in the cancer patients as they enter the hospital, changes in titer during the hospital stay and alteration as infection occurs or during immunosuppressive therapy. It is postulated that such changes may be an early indication of infection with specific microorganisms. A drop in antibody levels may also be an indication to initiate supportive therapy.

Methods Employed:

Standard techniques including immunoelectrophoresis.

Major Findings:

Collection and filing of stored sera continues. Microorganisms recovered from the patients which might be significant are also routinely collected for future antigen preparation. Further investigation is dependent upon availability of personnel.

Significance to Bio-medical Research:

To establish "normal" antibody levels of a particular patient. These are known to vary from person to person. To monitor changes in specific antibody titers for the purpose of more clearly delineating behavior of antibody levels during therapy or infections. To correlate the changes with presence of infecting organisms and determine if such changes can serve as an aid in the early detection of infection and thus serve as an indication for supportive therapy.

Proposed Course:

To proceed along the lines indicated.

Honors and Awards:

None

Presentations:

None

Publications:

None

Serial No.: NCI-6039
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NCI
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: A Computerized Program for Reporting Results of Clinical and Research Microbiology by a Laboratory Geographically Removed From the Patient Care Area

Previous Serial No.: NCI-6039

Principal Investigator: Gerald Vermeulen, M.D.

Other Investigators: Viola Mae Young, Ph.D., Richard Hsieh, Dr.PH

Cooperating Units: Health Services Research, USPHS Hospital, Baltimore, Maryland

Man Years: July 1, 1970 - June 30, 1971:

Total: 1-1/2
Professional: 3/4
Other: 3/4

Project Description:

Objectives:

1. To enlarge the capabilities of the microbiology final report generating program to make it possible to report the results of viral cultures. Serologic studies using any of several techniques, bacteriologic serologic determinations, environmental and laminar flow studies, and certain specific procedures such as electron microscopic studies of fluid from skin lesions.

2. Provide the necessary modifications in the error detecting program to process the data records generated as a result of the above changes.

3. To provide the necessary modifications to record the data developed as a result of the above changes onto the same, common data tape and to also retrieve this new information.

4. Incorporate an error detecting program with the ability to check for non-existent source, genus and species codes.

5. Develop methods of incorporating data generated by other service laboratories into the currently used system, by automated methods, insofar as possible.

Methods Employed:

Equipment used is as described in Project Report No. NCI-6039, "A Computerized Program for Reporting Results of Clinical and Research Microbiology By a Laboratory Geographically Removed from the Patient Care Area." In addition, the retrieval and other data processing programs are processed on the facilities of the Health Services Computer Center, Health Services Research, U.S. Public Health Service Hospital, Baltimore, Maryland, with the cooperation of Dr. Richard Hsieh, Chief. These facilities include three control data corporation computers, Model 1704, with disc storage device, associated tape drives, line printers, teletypes, card punching equipment, etc.

Major Findings:

The reporting system continues to operate successfully. An error detecting program detects errors of input data. A program for recording this information onto a permanent microbiology data tape and for its retrieval is in successful operation. The error detecting program detects format errors, including errors of patients' names and hospital numbers. This is possible by the use of a separate patient dictionary which contains this information. Data from deceased patients is later transferred to an inactive patient data tape.

Modifications have been made to use the system to report the results of laminar flow studies being conducted at the Clinical Center, N.I.H. This is also in operation and the data is being recorded onto a separate data tape which may also be retrieved.

A comprehensive microbiology code dictionary has been prepared which includes the possible source, genus and species codes for use with the program. It has various devisions to facilitate the use of the dictionary by clinical, laboratory, and other personnel. It will be indexed by related and synonymous terms, referring the user to the appropriate codes. It also contains an alphabetic listing of all codes with their respective meanings.

Significance to Bio-medical Research:

The system has been proven to provide the necessary flexibility yet is not excessively time consuming to operate nor is the operation too complex for most secretarial and laboratory workers to operate. It is now one of the few successful microbiology computer programs in operation. The success achieved is probably due to the use of letter codes for all clinical sources, genus and species names, plus the relatively simple fixed format of data input and storage. This also makes the subsequent retrieval programs less complex then would otherwise be necessary.

A prime advantage of the system is the ability to form a permanent data information bank, and the associated ability to retrieve the information by patient, source, results, date, etc. for research purposes.

Proposed Course:

1. Continued alterations of the system as necessary to provide for optimal operation and service to the clinical needs of the patient and physician.

2. Develop more methods to make it possible to have access to more data developed in the B.C.R.C. laboratory facilities as well related service facilities.

3. Incorporate more automated and more completed methods for error prevention and detection in input data. Increase the capability for data summary and processing for more specific purposes and objectives in a more usable format.

4. Develop statistical methods of analysis of antibiotic sensitivity test results and other data.

5. Make necessary changes to simplify the processing of final reporting, storage, and retrieval of the information on a single hardware system.

Honors and Awards:

Chosen for press release at the American Society of Microbiology Meeting, Boston, Mass., April 26, 1970.

Presentations:

1. Presented at the American Society of Microbiologists, Boston, Mass., April 26, 1970, "Use of Computers in Reporting Microbiologic Results By Contracting Laboratories," G. D. Vermeulen, V. M. Young, S. V. Schwab, R.K.S. Hsieh.

2. USPHS Clinical Society, Washington, D. C., April 2, 1970, "Use of Computer Facilities in the Monitoring of Microbiologic Flora of Cancer Patients," Gerald Vermeulen, M.D., Viola M. Young, Ph.D., Dolores Kenton, B. S., Marcia Moody, M.S., Stephen Schwab, B.A.

3. Clinical Microbiology Section, Eastern Pennsylvania Chapter of the American Society for Microbiology, Philadelphia, Pa., January 18, 1971, "The Use of Computers By Clinical Microbiologists," Viola Mae Young, Ph.D.

4. To be presented at the American Society of Microbiologists, Minneapolis, Minn., May 3, 1971, "Microbiologic Data Retrieval: Systems and Information Utilization," Gerald Vermeulen, V. M. Young, R.H. Hsieh.

Publications: None

Serial No.: NCI-6218
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: An Investigation of Klebsiellasp Isolated from Cancer Patients, their Serotype and Antibiograms

Previous Serial No: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Robert Good, Ph.D.

Cooperating Units: Hazelton Laboratories, Virginia

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/6
Professional: 1/6
Other:

Project Description:

Objectives:

Concurrently, with the initiation of the Carbenicillin Gentamicin Protocol in cancer patients for the purpose of control, particularly of Pseudomonas aeruginosa infections, an increase in the number of Klebsiella isolates was noted. The objective of this study is to serologically identify these Klebsiella strains to determine prevalence of a particular type and possible endemicity within the hospital environment. It is also planned to compare the results with the antibiograms of the strains.

Methods Employed:

Biochemical reactions were used to classify Klebsiella species. Isolates were typed with Difco specific antisera (capsular swelling) after transfers on Worfel-Ferguson agar and broth to promote good capsule formation.

Major Findings:

A total of 35 different serological types were recovered from 40 patients. No particular serotype was noticeably prevalent. Klebsiella type 18 was most frequently found, but colonization with this type was transient except in one case. Types 38,63,45 and 13 were next most frequently isolated. Eighteen other types occurred in one patient each. Length of the period of colonization varied. For example, patient J. D. carried a single type for

4 months during which time occasional minor variations in biochemical reactions were opened; patient R. L. over a 4 month period first shed type 53, then type 54, the latter resulting in bacteremia; patient B. G. harbored 7 different types some of which occurred simultaneously in different sites. The observed changes did not correlate with changes in the antibiogram. There was no evidence that a single serotype of Klebsiella was endemic or being spread within the environment.

Significance to Bio-medical Research:

The determination of prevalence of specific strains of resistant microorganisms within a hospital environment, the specific definition of a problem,is always the best first step towards its elimination. The possible relationship of specific types of Klebsiella to resistance to antibiotics would be helpful for therapeutic purposes.

Proposed Course:

Further serological studies will be done and an attempt made to correlate the additional data to define possible predictable patterns.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6219
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Proposed Title: The Detection of Pneumocystis carinii Infection in Cancer Patients

Previous Serial Number: Same

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Robert C. Good, Ph.D., Marcia R. Moody, M.S.

Cooperating Units: Hazelton Laboratories, Reston, Virginia

Man Years: (July 1, 1970 - June 30, 1971):

Total: 0
Professional: 0
Other:

Project Description:

Objectives:

Infection caused by Pneumocystis carinii is not an uncommon finding in cancer patients who are debilitated or immunosuppressed. Lung biopsy, the only procedure presently recognized to give a reasonably good possibility of establishing a diagnosis during life, is at best a risky procedure in a cancer patient undergoing chemotherapy. Several European publications report good serological results by use of a complement-fixation test. The objective of this study is to develop a serological or other procedure to detect infection.

Methods Employed:

Animals would be immunosuppressed for several weeks and given antibiotics during the interim to allow natural infection with Pneumocystis carinii to develop without demise of the animal. Most of the animals would then be sacrificed, their lungs excised and ground and the homogenized tissue centrifuged and washed repeatedly to provide a clean antigen preparation. Other animals will be allowed to recover and bled at intervals as a possible source of high titer antibody. Attempts will be made to obtain other high titer antisera to serve as a control. Several serological systems, including gel diffusion, complement-fixation and hemagglutination are to be used as possible serological means of checking for the presence of antibodies.

Major Findings:

None. The antigen preparation was delayed by lack of funds.

Significance to Bio-medical Research:

The differential diagnosis of this disease is most difficult in the absence of demonstrated organisms. It is important that this differentiation be made to allow properly definitive treatment against the protozoan.

Proposed Course:

Funding for the antigen has been requested. The future course of this project is dependent upon a good antigen.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6220
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Use of Time-Sharing Computer Facilities for Correlating Clinical and Patient Data with Microbiologic Research Studies

Previous Serial No.: Same

Principal Investigator: Gerald D. Vermeulen, M.D.

Other Investigators: Viola Mae Young, Ph.D.

Cooperating Units: N.C.I., N.I.H. (Barbara Murray)

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/6
Professional: 1/12
Other: 1/12

Project Description:

Objectives:

1. Establish data banks, using computer time-sharing facilities which contain patient information and vital statistics which may be accessed from both the hospital and distantly located contracting facilities for immediate use.

2. Provide a means of updating this data bank.

3. Establish a system of recording and retrieving B.C.R.C. patient census information.

4. Make this information available for use to others, either as part of programmed computer data processing systems, or by itself.

5. Continue to maintain close communication between the distant contracting microbiology laboratory and the clinician supplying patient care.

Methods Employed:

The system was originally designed as described in previous project report #6220. It has become subsequently necessary to modify the objectives to be more inclusive, as herein described.

The originally described hardware (CEIR - SYSTEM 420) was found to have inadequate systems commands and file capabilities. The operation has since been transferred to Call-A-Computer time-sharing facilities. Communications with this system are by teletype as previously described.

Major Findings:

Data files have been established containing names, hospital numbers of B.C.R.C. patients and other vital statistics. Subroutines in BITCAC programming language have been written which read, write, and delete the information in these files and may be used in other programs.

A program to record patient census information has also been written which updates the patient information files, records current B.C.R.C. patient census information, and ultimately records all census changes and enters and updates the patient information files.

Significance to Bio-medical Research:

The existence of the patient data files makes this information accessable to distantly located facilities. The recorded census information provides data in epidemiologic studies. This basic type of information, accessible by computer, provides the means for a large scale system of data reporting and processing needed by laboratory services geographically separated from the patient care area with a minimal duplication of effort.

Proposed Course:

The system will be expanded and further programs will be created to reduce the amount of time needed for recording and transmitting information between hospital and contracting laboratory.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI 6221
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: The Effect of Various Surfactant Chemical Agents on the Serum Sensitivity of Pseudomonas aeruginosa and the Influence of such Phenotypic Conversion on Host defense Mechanisms

Previous Serial Number: Same

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, PH.D.

Cooperating Units: None

Man Years (July 1, 1970 - June 30, 1971)

Total: 1/12
Professional: 1/12
Other:

Project Description:

Objectives:

The objective is to determine the effect of selected chemical agents on the cell wall of P. aeruginosa in vitro and in vivo and to determine if phenotypic alteration influences the host defence mechanism against this organism.

Methods Employed:

Serum bactericidal tests, antibiotic sensitivity tests, carbohydrate and lipid analyses by paper, thin layer and gas-liquid chromatography.

Major Findings:

It has been confirmed that EDTA alters the invitro sensitivity of P. aeruginosa to antibiotics.

Significance to Bio-medical Research:

Virulence of the bacterium and susceptibility to serum bactericidal activity of the host are related. The phenotypic conversion may enhance the host defence mechanism to P. aeruginosa.

Proposed Course:

Continuation of this project is dependent upon obtaining technical help for the principal investigator.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6222
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Studies on Pseudomonas aeruginosa in a Cancer Research Center, II. Comparison of Immunotypes from Cancer and non-Cancer Patients

Previous Serial Number: (consider in conjunction with Project #6030)

Principal Investigator: Marcia R. Moody

Other Investigators: Viola Mae Young, Ph.D., Gerald Vermeulen, M.D., Dolores M. Kenton, B.S., Myron Fisher, Ph.D.

Cooperating Units: Bacteriology Laboratory, Department of Pathology, USPHS Hospital, Baltimore, Md., Parke, Davis and Co., Detroit, Michigan.

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/3
Professional: 1/3
Other:

Project Description:

Objectives:

The objective of the second part of this study was to compare the Pseudomonas aeruginosa immunotypes recovered from cancer and non-cancer patients.

Methods Employed:

Serological agglutination tests using new antisera prepared from "protective" antigens and chi-square analysis of the resulting data.

Major Findings:

Pseudomonas aeruginosa was recovered from 78 hematological cancer patients, 53 solid tumor patients and 111 non-tumor patients. More of all patient groups were colonized with Pseudomonas immunotype 1 than any other type; patients with immunotype 2 were also quite common in the three groups. However, the incidence of immunotype 4 was significantly higher in hematological cancer patients ($p. < .05$). Certain immunotypes seemed to be more capable of establishing persistent colonization in the patient groups than others, i.e., immunotype 7 in hematological cancer patients, 6 in solid tumor patients and 3 and 6 in non-tumor patients.

Significance to Bio-medical Research:

This study revealed the prevalence of various immunotypes of P. aeruginosa among certain disease groups. These data provide basic information necessary for adequate Pseudomonas infection monitoring of certain high risk patients as well as for possible therapy with Pseudomonas immunoglobulin.

Proposed Course:

Project #6030 has been expanded to include several projects. The project of immunotyping has been discontinued for general hospital patients but is continued for cancer patients to monitor possible epidemics and their sounds. It also serves as a basis for evaluating the Pseudomonas immunoglobulin therapy.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6223
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Studies on Pseudomonas aeruginosa in a Cancer Research Center.
I. Distribution of Immunotypes from Human and Environmental Sources

Previous Serial Number: Same (Consider in conjunction with Project #6030 and with project entitled: "Use of Hyperimmune Globulin to Prevent Colonization and/or Infection with Pseudomonas and as an Adjunct in Treatment of Pseudomonas Infection")

Principal Investigator: Marcia R. Moody

Other Investigators: Viola Mae Young, Ph.D., Gerald Vermeulen, M.D., Dolores M. Kenton, B.S., Myron Fisher, Ph.D.

Cooperating Units: Bacteriology Laboratory, Department of Pathology, USPHS Hospital, Baltimore, Md., Parke, Davis and Co., Detroit, Michigan

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/2
Professional: 1/3
Other: 1/6

Project Description:

Objectives:

Pseudomonas aeruginosa is a frequent cause of infection in immunosuppressed cancer patients. As it is also resistant to therapy with antibiotics, it is the single most common cause of infectious mortality in these patients. The objective of Part I of this study was to determine the distribution of Pseudomonas immunotypes in cancer and non-cancer patients to establish basis for future therapy of cancer patients with Pseudomonas immunoglobulin.

Methods Employed:

Serological typing using new antisera prepared from "protective" antigens.

Major Findings:

Analysis of 755 Pseudomonas aeruginosa strains recovered from cancer and non-cancer patients revealed the following data: Colonization of the oral-respiratory tract of patients by immunotypes 3 and 6 was significantly more frequently than the other immunotypes ($p < .001$ and $p < .01$). Immunotype 1 was more likely to be recovered from wounds than from blood ($p < .01$). All immunotypes were frequent colonizers of the GI tract; patient incidence ranged from 48% to 60%. Approximately 25% of the patients who were colonized by immunotype 2,4, and 7, had bacteremic episodes, and 20% of the patients had 1,5, and 7 recovered from wound cultures. Certain immunotypes were more capable of establishing extended colonizations in various areas. Immunotypes 6 and 7 persisted in sputa and nares more often than the other types; the former also caused protracted colonization of the urine whereas the latter type demonstrated sequent colonization of the feces, blood, and the mouth. Immunotype 1 was also able to maintain longer colonization of the mouth.

Significance to Bio-medical Research:

This study revealed the prevalence of various immunotypes of Pseudomonas and the low number of untypable strains in the patient population. This provides the basic information necessary to possible therapy with immunoglobulin.

Proposed Course:

Routine monitoring of cancer patients samples and their environment for Pseudomonas and immunotyping of strains recovered will be continued.

Honors and Awards: None

Presentations:

"Distribution of Pseudomonas aeruginosa immunotypes in a Cancer Research Hospital" Microbiology Seminar given at University of Maryland, Department of Microbiology, School of Medicine.

Publications: None

Serial No.: NCI-6224
1. BCRC
2. Baltimore, Md.
3. Clinical Branch

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Title of Project: Studies on Pseudomonas aeruginosa in a Cancer Research Center. III. Hemagglutinin Levels to Pseudomonas aeruginosa in Cancer Patients

Previous Serial Number: Same (Consider in conjunction with Project #6030 and with projects entitled: "II. Comparison of Immunotypes from Cancer and Non-Cancer Patients" and I. Distribution of Immunotypes from Human and Environmental Sources")

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D., Gerald Vermeulen, M.D., Dolores M. Kenton, B.S., Myron Fisher, Ph.D.

Cooperating Units: Bacteriology Laboratory, Department of Pathology, USPHS Hospital, Baltimore. Md., Parke, Davis and Co., Detroit, Michigan.

Man Years (July 1, 1970 - June 30, 1971):

Total: 0
Professional: 0
Other:

Project Description:

Objectives:

Pseudomonas aeruginosa is a frequent cause of infection in immunosuppressed cancer patients. As it is also resistant to therapy with antibiotics, it is the single most common cause of infectious mortality in these patients. The objective was to ascertain the immune responses of cancer patients colonized by or infected with P. aeruginosa as compared with those of patients from whom no Pseudomonas strains were recovered.

Methods Employed:

Microtiter hemagglutination method using 7 purified "protective"antigens.

Major Findings:

Further hemagglutination tests were not performed during the past year due

to lack of technical help. An automated Microtiter system has been purchased and is being tested to achieve greater efficiency and overcome the obstacle of personnel shortage. A number of antigens have been prepared and sera collected for future testing.

Significance to Bio-medical Research:

The data obtained in this study will be used as a baseline in the investigation of antibody levels in patients who received therapy with Pseudomonas immunoglobulin monitoring. It also provides information relating serum antibody levels to subsequent colonization and/or infection.

Proposed Course:

The present project will be continued to provide adequate monitoring of immune reresponses as an adjunct to bacteriological investigation.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6251
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Relationship of Pseudomonas aeruginosa Immunotypes to Antibiotic Sensitivity Patterns

Previous Serial Number: None

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola M. Young, PH.D., Stephen Schimpff, M.D., Dolores M. Kenton, B.S.

Cooperating Units: None

Man Years (July 1, 1970 - June 30, 1971)

Total: 1/6
Professional: 1/6
Other:

Project Description:

Objectives:

Sequential cultures from cancer patients are taken to monitor colonization and/or subsequent infection with Pseudomonas aeruginosa. It was noted that occasional changes in antibiograms occurred in strains recovered from these patients. It is frequently assumed that a single species of microorganism recovered from a patient, either sequentially from the same site or from multiple sites, were the same strain if the antibiograms did not vary; conversely it is assumed that the strains of microorganism were different if the antibiograms did vary. As intraspecies differentiation of P. aeruginosa can now be readily determined by serological means, a study was undertaken to ascertain the correctness of such assumption, i.e., the possible relationships between the immunotype of P. aeruginosa and their antibiogram including changes as one immunotype was replaced by another.

Methods Employed:

Intraspecies serological typing with "Fisher" antisera. Antimicrobial susceptibility testing by the Bauer-Kirby-Sherris-Turck method.

Major Findings:

Consistent results for all immunotypes were obtained from certain anti-

biotics. All were resistant to cephalothin and ampicillin and largely resistant to kananycin and tetracycline as well as susceptible to colistin, polymixin B and carbenicillin. However, there was considerable variation in susceptibility to the other agents tested. No gentamicin resistant strain was found but 5 to 32% of the strains, depending on the immunotype, were moderately susceptible. More strains of immunotype 4 were susceptible to chloramphenicol and resistant to neomycin and more strains of immunotype 5 were resistant to streptomycin and moderately susceptible to gentamicin than the other types. Immunotypes 3 and 6 had the highest percentages of strains that were susceptible to gentamicin while the latter had the largest number of chloramphenicol resistant strains. Although in vitro changes in susceptibility patterns of a patient's isolates did occur, there was no correlation between these changes and the length of colonization or the site of infection. Neither did the presence of another colonizing immunotype influence the variations in antibiograms. Therefore a change in antibiogram did not necessarily indicate a particular immunotype nor did the same antibiogram indicate the strains were of the same intraspecies identity.

Significance to Bio-medical Research:

The assumption that variation in antibiograms of P. aeruginosa reflects an intraspecies difference that may parallel antigenic variation has been disproved. As the antibiogram changes were generally not permanent these changes do not offer a valid means of tracing strains of P. aeruginosa within an environment.

Proposed Course: This project has been completed.

Honors and Awards: None

Presentations:

10th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., October, 1970.

Publications:

Accepted for publication in Antimicrobial Agents and Chemotherapy - 1970-1971.

Serial No.: NCI-6252
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Phagocytosis of Pseudomonas Aeruginosa by Immune and Non-immune Guinea Pig Macrophages

Previous Serial No.: None

Principal Investigator: Marcia R. Moody, M.S.

Other Investigator: Viola Mae Young, Ph.D.

Cooperating Units:

Man Years (July 1, 1970 - June 30, 1971)

Total: 1/3
Professional: 1/3
Other:

Project Description:

Objective:

Pseudomonas aeruginosa is a frequent cause of infection in immunosuppressed cancer patients. As immunization with a vaccine or hyperimmune globulin fractions are under investigation as a means to control pseudomonas infections, it is important to determine if the immune state influenced the phagocytosis of the bacterium by macrophages. An investigation was undertaken to establish the possible effect of the immune state on the rate of phagocytosis.

Methods Employed:

Peritoneal macrophages are obtained from normal adults guinea pigs which have no detectable antibodies against the 7 P. aeruginosa immunotypes and from adult guinea pigs which have been actively immunized with one of the immunotypes. Various concentrations of this immunotype are incubated with the macrophages for a set time period in Leighton tubes with coverslips. Coverslips are removed, stained with May Grunwald-Giesma stain and fixed to glass slides. The stained macrophages are examined for engulfed bacteria and phagocytic index determined.

Major Findings:

Project in progress.

Significance to Bio-medical Research:

As Pseudomonas aeruginosa causes an unusual number of fatal infections in leukemia patients it is important to understand the possible host defense mechanisms against this organism. Such information could lead to enhancement of defense and lowered infection fatalities in this patient group.

Proposed Course:

To carry out the investigation along the lines indicated.

Awards and Honors: None

Presentations: None

Publications: None

Serial No.: NCI-6253
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Incidence of Pseudomonas species other than aeruginosa in Cancer Patients

Previous Serial Number: None

Principal Investigator: Marcia R. Moody, M.S.

Other Investigators: Viola Mae Young, Ph.D. and Dolores M. Kenton, B.S.

Cooperating Units: None

Man Years (July 1, 1970 - June 30, 1971)

Total: 1/3
Professional: 1/3
Other:

Project Description:

Objectives:

Bacteria of the genus Pseudomonas that are not biochemically identical to the species aeruginosa have been recovered from cancer patients and their environment. The importance of colonization of immunosuppressed patients with these microorganisms has not been evaluated. Definitive criteria for identification of some of these species has not been established. Several schemata have been devised, but not adequately compared. This investigation was undertaken to: 1) compare the various schemata, evaluate their usefulness, and establish optimal means of differentiating these species; 2) ascertain the identity of the isolates according to the classification scheme which we have developed and evaluated; 3) determine the importance of these microorganisms to cancer patients.

Methods Employed:

Known strains of the various species of Pseudomonas (other than aeruginosa) have been obtained from the American Type Culture Collection. These strains are being run as controls, together with a great many non-aeruginosa isolates from cancer patients according to the biochemical schemata of Stanier (based on utilization of various substrates in a minimal medium), Hugh (based on oxidative vs fermentative reactions) and Pickett (similar to that of Hugh, utilizing buffered media.)

Major Findings:

The Project is in progress. It is apparent that the various schemata do not all give parallel results.

Significance of Bio-medical Research:

As increasing numbers of patients are immunosuppressed or otherwise have lowered host resistance, microorganisms which are usually considered "non-pathogens" and which colonize such patients must be studies and evaluated to determine their possible importance. The present study will attempt to establish guidlines for identification to make such an evaluation possible for a group of microorganisms which has been found in considerable numbers in samples from cancer patients.

Proposed Course:

The project will continue until the problems outlined have been resolved.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6254
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: A Comparison of Buffered Glycerol Saline, Todd-Hewitt Broth and Unpreserved Fecal Material as the Means of Sample Collection to Determine Fecal Microbial Flora

Previous Serial No.: None

Principal Investigator: Gerald Vermeulen, M.D.

Other Investigator: Viola Mae Young, Ph.D.

Cooperating Units: None

Man Years: (July 1, 1970 - June 30, 1971):

Total: 1/12
Professional: 1/12
Other:

Project Description: None

Objectives:

The study was undertaken to determine the optimal method of sampling the total bacterial flora of the feces of cancer patients.

Methods Employed:

Duplicate rectal swabs are taken from patients. One specimen is placed into Todd-Hewitt Broth and the other is placed into buffered glycerol saline. Stool samples are also aliquoted into the same two types of holding medium, in addition to retaining an unpreserved portion. All specimens are processed in the same way, identifying all isolates of the sample. The source codes assigned to the specimen for computer processing indicate which aliquot was cultured. Retrieval of the information by source code from the computerized information system supplies the accumulated information which is summarized.

Major Findings:

It appears that the best method of a total sampling of the flora is by collection of stool from the patient; however, the described methods of rectal swab sample collection and preservation is satisfactory for most microorganisms and no particular method is advantageous for all microbial

species. It appears that the viability of Pseudomonas species and Staphylococcus aureus are maintained best in Todd-Hewitt Broth. Some species are recovered but rarely from any form of preserved fecal material.

Significance to Bio-medical Research:

The increasing incidence of nosocomial infections and endogenous infections by antibiotic resistance microorganisms usually believed to be of little pathogenic potential makes it necessary to determine the optimal methods of isolation of these microorganisms from culture specimens containing mixed flora. Most studies to determine optimal methods of preservation of stool flora have been done in search of the usual enteric pathogens, i.e., Shigella and Salmonella species. Particularly in immunosuppressed and antibiotic treated patients and patients being studies in laminar flow "life island" conditions, it is important to know if the methods used in taking and preservation of specimens are suitable to allow the less commonly encountered opportunistic microorganisms to be isolated, as well as the more common enteric pathogens.

Proposed Course:

The collection of duplicated specimens will be continued over an extended period of time. Statistically valid results will require large numbers of such comparisons, and this will be done with the aid of the computerized methods of recording and retrieval of the information.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6255
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: A Study of the Incidence and Types of Gram Negative Rods and Other Pathogenic Microorganisms in the Oral Cavity of B.C.R.C. Patients in Relationship to the Dentition

Previous Serial No.: None

Principal Investigator: Gerlad Vermeulen, M.D.

Other Investigator: Viola M. Young, Ph.D.

Cooperating Units: None

Man Years (July 1, 1970 - June 30, 1971):

Total: 1/6
Professional: 1/12
Other: 1/12

Project Description:

Objectives:

The objective is to determine the types and incidence of gram negative rods and other pathogenic microorganisms in the oral cavity of tumor patients and, if possible, to relate their presence with the dentition of the patient.

Methods Employed:

Gingival and mouth cultures are taken when ordered by the physician and as part of a surveillance study of all B.C.R.C. patients. The specimens are coded as to the presence or absence of natural teeth or dentures at the time the cultures are taken. This information is then retrieved by computer methods after routine processing by the microbiology laboratory. The information is summarized as to microorganisms isolated and the dental status at the time the culture was taken.

Major Findings:

The presence of natural teeth and/or dental pathology is not the only factor fostering the colonization of enteric and other pathogenic microorganisms in the mouth, as is often believed. It does appear that the absence of natural teeth with or without dentures is associated with colonization of

the mouth and gingiva more commonly with certain specific microorganisms. More data are necessary to confirm these presumptive findings.

Significance to Bio-medical Research:

The significance of pathogens in the oral cavity and specifically in the gingiva of cancer patients is not clearly defined, however, individual patients have given evidence that this area may be a source of the agents causing more serious generalized infections. The fact that pathology does not need to be present to harbor gram negative rods in the gingiva is not usually understood and becomes of importance particularly in patients studied under laminar flow surroundings. This is also important in patients susceptible to infection due to their basic disease and/or immunosuppression.

Proposed Course:

This project will be continued along the same lines.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6256
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Viral Diagnostic Services for Clinical and Research Studies for Laminar Flow Study Patients at B.C.R.C. and at N.I.H., Clinical Center by Contracted Laboratory Facilities

Previous Serial No.: None

Principal Investigator: Viola Mae Young, Ph.D.

Other Investigators: Gerald Vermeulen, M.D., Dolores M. Kenton, B.S.

Cooperating Units: Viral Diagnostics Laboratory, Georgetown University Hospital, Georgetown University, Washington, D.C.

Man Years (July 1, 1970 - June 30, 1971):

Total: 2/12
Professional: 2/12
Other: None

Project Description:

Objectives:

1. To supply virologic diagnostic services of a high quality for the study of actual and potential infections of cancer patients and patients being studied under the Laminar Flow Study Project.

2. To provide information on the occurrence and nature of viral agents in the environment which may be potentially agents of disease in cancer and Laminar Flow study patients.

3. To study the effect of a Laminar Flow environment upon the incidence and type of viral infections in cancer patients.

4. To investigate the role of viral agents in the total microbiologic milieu of cancer patients.

Methods Employed:

Samples for viral culture and other studies and patient serum samples for detection of viral antibodies are transported to the processing laboratory either in the original form or frozen at - 70 degrees centigrade in viral isolation broth by chauffeur driven government vehicle. Specimens are taken

on a daily basis or as the need dictates. The same driver also transports the specimens collected at N.I.H. as part of their laminar flow study project. The material is processed by the laboratory and preliminary results reported by teletype using commercially operated telephone lines. The information received at the hospitals is then made known to the physicians responsible for patient care.

The final results will be reported by the means of a computer program and Microbiological Reporting System which has been described in the project, "A Computerized Program for Reporting Results of Clinical and Research Microbiology By a Laboratory Geographically Removed From the Patient Care Area," after appropriate modifications have been completed to accommodate viral study results.

Major Findings:

The project has just been initiated. A limited number of clinical samples have been studied which have already given indications that a significant number of infectious complications occurring in leukemia patients may be of viral etiology. Information gained from serologic studies of patients suspected of viral infections has also been of clinical importance particularly during the course of a number of Herpes infections which recently occurred among immunosuppressed patients at the B.C.R.C.

Significance to Bio-medical Research:

Proposed Course:

L. To provide continued support in the care of cancer patients.

2. To provide continued investigation of the types, rates of occurrences, and significance of viral infections in laminar flow and cancer study patients as outlined in "Objectives."

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6257
1. BCRC
2. Clinical Branch
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: A Study of Relative Effectiveness of Different Types of Blood Culture Media and Laboratory Techniques for the Isolation of Specific Types of Microorganisms

Previous Serial No.: None

Principal Investigator: Gerald Vermeulen, M.D.

Other Investigator: Viola M. Young, Ph.D.

Cooperating Units: None

Man Years: (July 1, 1970 - June 30, 1971):

Total: 1/6
Professional: 1/6
Other:

Project Description: None

Objectives:

1. To study the effectiveness of various types of blood culture media in the isolation of different types of microorganisms.

2. To compare, concurrently, the possible effects of minor variations of laboratory technique upon the ability to isolate these microorganisms.

Methods Employed:

The types of blood culture media available for use are tryptic soy broth (Difco prepared bottles,) brain heart infusion (Difco prepared bottles,) thiol, (Difco prepared bottles,) brain heart infusion with cysteiene under nitrogen, and thioglycollate broth.

Blood cultures submitted for study are coded by source indicating the specific type and number of media inoculated with the original specimen. This information is then retrieved from the computer microbiology data tape and summarized. Similar information has been gathered from other bacteriology laboratory facilities available to B.C.R.C. patients.

Major Findings:

Some microorganisma are readily isolated from all types of media, particularly Pseudomonas. Anaerobic gram negative rods are usually able to be isolated from thiol media, however, it appears that multiple types of media are necessary for optimal results.

Some evidence indicates that similar laboratory techniques performed in the two laboratories give small but significant differences in recovery rates for some types of organisms. The possible reasons are being investigated.

Significance to Bio-medical Research:

Proposed Course:

The proposed course is to proceed with the collection of this type of information,as a continuing method of evaluation of the laboratory and media available.

Honors and Awards: None

Presentations: None

Publications: None

Serial No.: NCI-6228
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Preliminary Clinical and Pharmacological Evaluation of Camptothecin Sodium NSC 100,880

Previous Serial Number: Same

Principal Investigator: Howard M. Shapiro, M.D.

Other Investigators: A. M. Guarino, M.D., V. T. Oliverio, M.D., J. B. Block, M.D.

Cooperating Units: Laboratory of Chemical Pharmacology, NCI

Man Years:

Total:	3/4
Professional:	3/4
Other:	0

Objectives:

To determine the appropriate dosage schedule for this new anti-tumor agent and to carefully observe the toxicities it produces. To determine the anti-tumor spectrum of this drug and compile accurate data regarding its palliative effects. To determine the drug's pharmacological properties.

Methods Employed:

Patients are given camptothecin sodium intravenously at varying dosage levels over varying periods of time. Plasma, urine, other body fluids and tissues are collected at various times and analyzed for camptothecin levels, using a fluorometric spectrophotometric method highly specific for camptothecin.

Major Findings:

1. A dose of 7.5 mg/Kg given I.V. push over 5 to 10 minutes every 2 to 3 weeks is well tolerated. Higher doses produce too great a granulocytopenia and thrombocytopenia to be useful, while lower doses have not produced appreciable tumor response. Bi-weekly doses of 25 and 37.5 mg/M^2 have not produced responses; 50 mg/M^2 may produce a response but toxicity is appreciable.

Major Findings (cont'd):

2. Camptothecin is neither as active against gastrointestinal cancer nor as non-toxic as was originally thought--the response rate observed here now being 4 responses in 17 patients. Alopecia, nausea, vomiting and hemorrhagic cystitis have occurred in many of the patients treated, in addition to marrow depression.

3. Camptothecin has an initial rapid plasma disappearance followed by a slower rate. No drug is detectable in urine or plasma beyond the sixth day in patients with normal renal function. Up to 40% of unmetabolized drug can be found in the first 48-hour urine collection. No drug has yet been detected in body tissues.

Significance to Cancer Research:

Camptothecin appears to be a powerful mitotic inhibitor with some activity against gastrointestinal tract neoplasms, which are relatively unresponsive to chemotherapy. In combination with other drugs, this agent may be able to increase the success rate in the treatment of these lesions. The effectiveness of camptothecin against other tumors remains to be determined.

Proposed Course:

Camptothecin will be studied in combination with 5-fluorouracil and other agents against gastrointestinal tract tumors, but emphasis will be shifted toward determining its efficacy in leukemias, lymphomas, and other ordinarily sensitive tumors which have become refractory to other drugs.

Publications: None

Serial No.: NCI-6230
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Diagnostic Laparotomy and Splenectomy for Staging Hodgkin's Disease

Previous Serial Number: Same

Principal Investigator: Irwin Zarembok

Other Investigators: John Sutherland, Harold E. Ramsey

Cooperating Units: Pathology and Tumor Surgery Depts., USPHS Hospital

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives:

Accurate determination of the extent of lymphomatous involvement with Hodgkin's disease is important for the individual patient's prognosis and therapy. Valid assessment of the efficacy of different therapeutic modalities used in a population with Hodgkin's disease is also dependent upon a precise localization of disease prior to therapy. The purpose of this study was to determine more accurately the extent of abdominal disease by performing diagnostic laparotomy.

Major Findings:

Over 45 patients with Hodgkin's disease have undergone laparotomy and splenectomy for staging. The preoperative assessment of splenic involvement was incorrect in 1/3 of the patients studied. Many spleens of normal size were found to be involved and some large spleens were free of disease. Some positive lymphangiograms could not be confirmed at surgery. Involvement of nodes in the splenic hilum, porta hepatis and mesentery was demonstrated in many patients with extensive abdominal disease. Hepatic involvement has been found despite normal liver function tests and scans. There has been no operative mortality and minimal morbidity. Tolerance to extended field radiotherapy and combination chemotherapy has probably improved as a result

Major Findings (Cont'd):

of splenectomy.

Proposed Course:

Continue investigation.

Honors and Awards: None

Publications: None

Serial No: NCI-6259
1. BCRC
2. Medicine Service
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Conversion from the Traditional (or Source Oriented) to the Problem Oriented Medical Record; in paper form and computer form

Previous Serial Number: None

Principal Investigator: Robert J. Esterhay, Jr., M.D.

Other Investigators: None

Cooperating Units: PROMIS Laboratory
Department of Medicine
University of Vermont
Burlington, Vermont 05401

Telephone: (802) 865-7441 Ext. 421

Cooperating Investigators: Lawrence L. Weed, MD.
Laura B. Weed, M.D.
George E. Nelson, M.D.
Jan R. Schultz, Research Associate
Stephen V. Cantrill, Research Programmer
Keith G. Morgan, Research Programmer

Man Years:

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives:

To convert the traditional or source oriented medical records at BCRC to the Problem Oriented Medical Record, in paper form and concurrently to computer form utilizing the PROMIS laboratory computer system via telephone line with a touch sensitive cathode ray tube terminal that allows structured input by a directly interfaced medical user (in particular the physician and the nurse.)

Methods Employed:

The system whether in paper or computer form requires medical data to be collected, filed and identified with respect to a given problem and not to the source of the data as in the "traditional" record.

Major Findings:

The paper version of the Problem-Oriented Medical Record is now totally operational for all services, at BCRC, in-patient and out-patient. It has improved the quality of patient care at BCRC.

Significance to Cancer Research:

The system encourages the physician to use sound logic in his thoughts about patients--not only the cancer problem, but all problems--medical, social, psychiatric and demographic. The system allows the physician to communicate to all others who have immediate care of the patient. Inherent in the system is self-continuing education of the physician and all who assist in the care of the patient. An attending physician on the cancer service using the system can check a patient's problems and check the clinical associate's ability to collect data, interpret data, to develop a proper plan of treatment and to observe patients properly. Clinical investigation requires excellent records. The system makes retrospective review of data easier since data is in sequence by problem. When the computer form becomes implemented, clinical research information (for example, flow sheets) will be a by-product of patient care as opposed to an additional recording task. All of the above reasons will enable the physician to improve the care of his patients, improve his learning and communications to others about his patient's, and to collect more accurate clinical research data since a patient's problems can be looked at in context to each other and in proper time relationships.

Proposed Course:

The computer form of the system is to be implemented next at BCRC.

Honors and Awards: None

Publications: None

Serial No.: NCI-6260
1. BCRC
2. Medicine Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of the Efficacy and Toxicity of Daunomycin in a 180 mg/M^2 single IV Injection in the Treatment of Acute Non-Lymphocytic Leukemia

Previous Serial Number: None

Principal Investigator: William Greene

Other Investigators: David Huffman, Nicholas Bachur, Stephen Schimpff, Jerome Block

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description:

Objectives:

Daunomycin has been proven to be a potent antileukemic agent in patients with acute non-lymphocytic leukemia utilizing a dosage of 60 mg/M^2 IV for 3 successive days. Pharmacokinetic considerations, i.e., the prolonged serum half-life of daunomycin in a single intravenous injection as determined in patients with solid tumors, led to the institution of the regimen under study. The purpose of this study was to determine the toxicity and efficacy of the single dose in patients with acute non-lymphocytic leukemia, both untreated and previously treated.

Major Findings:

1. Results - Thus far, 17 patients have received at least one dose of daunomycin at 180 mg/M^2.

   Of the 17 patients, 12 were previously untreated and 5 were previously treated with other agents. Among the 12 patients not previously treated, 2 achieved complete remission, 2 achieved partial remission, 6 died during the course of therapy and 2 were considered failures. Among the 5 pa - tients previously treated with other agents, 2 achieved complete remission,

1 partial remission, 1 died in therapy, and 1 failed to receive adequate course before being changed to other agents.

2. Toxicity - The major toxicity was marked bone marrow depression, requiring in excess of 10-14 days for recovery. Deaths during therapy were primarily infectious. Three patients died with clinical evidence of congestive heart failure and shock, not conclusively linked to a known etiology.

Significance to Cancer Research:

Daunomycin is clearly an effective agent in some patients with acute non-lymphocytic leukemia. This regimen is a very toxic one, but may be of value in patients resistant to other agents, or among patients in whom precautions (LAF, bowel sterilization) are taken to prevent infection.

Proposed Course: Continue present study.

Honors and Awards: None

Publications: None

Serial No.: NCI-6261
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hyperalimentation in Patients with Malignant Disease

Previous Serial Number: None

Principal Investigators: Howard M. Shapiro, M.D., Clarence L. Fortner, M.S.

Other Investigators: Robert S. Benjamin, M.D., James C. Cradock, M.S., Lois Jepsen, B.S.

Cooperating Units:

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives:

To assess the usefulness of parenteral hyperalimentation as an adjunct to chemotherapy in the treatment of neoplastic disease.

Methods Employed:

Patients are given intravenous infusions of hypertonic glucose, protein hydrolysate, vitamins, and minerals providing up to 5500 calories daily. The solutions are administered through indwelling catheters placed in the subclavian vein using strict aseptic techniques. Metabolic balance is studied by chemical analysis of collected blood and urine specimens, and body weight is followed as well.

Major Findings:

Hyperalimentation appears to prevent weight loss in patients made anorexic by chemotherapy. Fluid imbalance and infectious complications from the intravenous catheter have occurred in about half the patients, but the incidence has decreased as the staff has gained experience with the technique. Long-term weight gain has been produced in several patients. The administration of high-calorie, high-nitrogen infusions has not resulted in demonstrable

Major Findings (cont'd):

acceleration of tumor growth.

Significance to Cancer Research:

Hyperalimentation has been reported to improve tolerance to chemotherapy in patients with advanced cancer; it is also likely that it will permit more extensive surgery to be done on patients with malignant disease. The demonstration that positive nitrogen balance and weight gain may be produced in man by a chemically defined, parenterally administered solution should open the way for many studies of the metabolism of patients and their tumors. This work may also be expected to better elucidate the causes of the cachexia associated with neoplasia.

Proposed Course: Continue present studies

Honors and Awards: None

Publications:

1. Fortner, C. L., Foley, T., and Cradock, J. C.: Reduction in serum CO_2 content during hyperalimentation therapy. Amer. J. Hosp. Pharm. 28: 121-122, 1971.

Serial No.: NCI-6262
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Initial Clinical Trials with Guanazole

Principal Investigator: Jerome B. Block

Other Investigators: S. Schimpff, I. Zarembok, R. Adamson

Cooperating Units: Laboratory of Chemical Pharmacology, NIH

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Objectives:

To study the metabolism of single intravenous doses and 24-hour infusions of guanazole, and to establish a tolerated intravenous dose level, toxicity and possible therapeutic effectiveness of guanazole in the treatment of malignant disease.

Major Findings:

Initial studies of C^{14} guanazole administration to patients with malignant disease undergoing therapy with guanazole has indicated that urinary excretion is rapid and complete following pulse I.V. dosage. The half-life is approximately 2 hours and excretion in the urine approaches 90% of the administered dose at 48 hours. Based on these findings, a dose schedule consisting of 5 consecutive 24-hour infusions of guanazole has been adopted. Excretion studies with C^{14} guanazole will be carried out on days 1 and 5 of this schedule and possible therapeutic efficacy will be assessed.

Proposed Course: Continue investigation

Honors and Awards: None

Publications: None

Serial No.: NCI-6263
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Protocol for Staging and Treatment of Lymphosarcoma and Reticulum Cell Sarcoma

Previous Serial Number: None

Principal Investigator: Robert S. Benjamin, M.D.

Other Investigators: Peter H. Wiernik, M.D.

Cooperating Units: None

Man Years:

Total: 1/4
Professional: 1/4
Other:

Project Description:

Objectives:

1. To improve staging procedures
2. To assess value of "prophylactic" splenectomy
3. To determine if localized disease is curable
4. To evaluate various therapeutic methods for generalized disease

Methods Employed:

Staging to include laparotomy and splenectomy in patients not definitely staged as IV by less invasive procedures. Randomization of patients with stage IV disease to prophylactic splenectomy or not. Randomization of stage I and II patients to radiation with or without subsequent chemotherapy. Randomization of stage III and IV patients to MOPP (HN_2, 6 mg/M^2 IV, day 1 and 8; VCR, 1.4 mg/M^2→ maximum 2 mg IV, day 1 and 8; procarbazine 100 mg/M^2 p.o. x 14; prednisone, 80 mg/M^2 p.o. q.o.d. every other course) or COP (Cytoxan 1500 mg/M^2 IV, day 1, VCR, 1.4 mg→ maximum 2 mg and prednisone 80 mg/M^2 p.o. q.o.d. every other course). Randomization of patients achieving CR to maintenance with procarbazine, 150 mg/M^2 p.o. q.d. or no maintenance. Use of cytocentrifuge to detect early CNS involvement and separate chemotherapy for those patients: COPP-B - Cytoxan, 1500 mg/M^2 IV, day 1, VCR, 1.4 mg/M^2→ maximum 2 mg IV, day 1 and 22, prednisone, 80 mg/M^2 p.o. q.o.d. x 11 doses,

Methods Employed (cont'd):

procarbazine, 100 mg/M^2 p.o. q.d. x 7, and BCNU, 80 mg/M^2 IV, days 2, 3 and 4.

Major Findings:

In the first 15 patients, the additional procedures advanced the stage of 3 patients from that originally determined by routine clinical evaluation.

There have been too few patients to date to assess the value of splenectomy or the two programs of chemotherapy.

Significance to Cancer Research:

To enhance our knowledge of the nature and extent of untreated lymphomas; to define the therapeutic role of prophylactic splenectomy and to further evaluate 2 combinations of chemotherapeutic agents, one of which has not been thoroughly investigated in this dosage schedule.

Proposed Course: Continue the study.

Honors and Awards: None

Publications: None

Serial No. NCI 6264
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical Observations of Infectious Diseases in Cancer Patients

Previous Serial Number: None

Principal Investigator: Stephen Schimpff

Cooperating Units: Research Microbiology

Man Years

Total:	5/16
Professional:	1/4
Other:	1/16

Project Description:

Objectives:

Infection is the most common major complication of cancer drug therapy and is a frequent complication in the untreated cancer patient. This investigation is aimed at 1) enumerating the incidence of the various types of infection in cancer patients, 2) describing and analyzing the clinical manifestations of infection, 3) assessing the value of various diagnostic maneuvers, and 4) correlating the site of infection, occurrence of bacteremia, etiologic organism, and clinical presentation and course with the patient's tumor type and defense mechanisms (granulocyte count, corticosteroid therapy, obstruction by tumor, etc.).

Methods:

Each patient with a known or suspected infection is seen by the principal investigator who examines the patient and reviews the chart, both initially and throughout the course of the infection. Detailed records of history, physical findings, nursing notes, laboratory data, and microbiologic isolations are kept. Monthly summaries are prepared and distributed. A detailed summarization will be prepared in July 1971 following the completion of the first year's observations.

Major Findings:

Detailed evaluation will not be available until after the end of the first year of study. Preliminary data suggest the following: 1) infection, especially by gram negative organisms, is common and is more prevalent than generally appreciated. 2) Infection can usually be related to the granulocytopenia of cancer drug therapy but 3) many patients develop infection regardless of the white blood cell level. These infections are usually due to a) tumor obstruction (e.g., bronchus or ureter) or b) iatrogenic maneuvers (e.g., urinary or intravenous catheters). 4) Most infections are of endogenous origin but the organism producing the infection may not have become endogenous until after hospital admission. 5) All patients with cancer are not equally susceptible to all infections but each has a specific set of predisposing factors which, once known, can be useful in predicting the subsequent types of infection which may occur. 6) Infection occurring in the granulocytopenic patient, even when caused by a common pathogen, will not present in the classical manner (e.g., rectal abscesses are watery, pneumonias less well defined on x-ray, etc.).

Proposed Course: Continue investigation.

Significance to Cancer Research:

A more complete understanding of the infectious problem in cancer patients is necessarily the first step in adequate prevention and therapy of infection.

Honors and Awards: None.

Presentations:

1. Relationship of Colonization with Pseudomonas aeruginosa to Development of Pseudomonas Bacteremia in Cancer Patients. Tenth Interscience Conference on Antimicrobial Agents and Chemotherapy. October, 1970. Chicago, Ill.

2. Empiric Use of Carbenicillin and Gentamicin for Presumed Infection in Granulocytopenic Cancer Patients. Eastern Section of the American Federation for Clinical Research. December, 1970. New York, New York.

3. Epidemic Varicella-Zoster Infection in Cancer Patients. 52nd Annual Meeting of the American College of Physicians. March, 1971. Denver, Colorado.

Publications:

Schimpff, S., Moody, M., and Young, V.M.: Relationship of Colonization with Pseudomonas aeruginosa to Development of Pseudomonas Bacteremia in Cancer Patients. Antimicrobial Agents and Chemotherapy - 1970. (in press).

Serial No. NCI 6265
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical Trials of Polyvalent Pseudomonas Hyperimmune Globulin

Previous Serial Number: None

Principal Investigator: Stephen Schimpff

Other Investigators: William Greene, Viola M. Young, Marcia Moody

Cooperating Units: Myron W. Fisher, Parke Davis and Company, Detroit, Mich.

Man Years

Total:	1 1/8
Professional:	1
Other:	1/8

Project Description:

Objectives:

Pseudomonas aeruginosa is the single most common cause of major infection at the Baltimore Cancer Research Center. Despite vigorous attempts to define and reduce environmental spread and despite the advent of effective antibiotic therapy, pseudomonas infections persist. Vaccination against the seven common immunotypes of Pseudomonas aeruginosa has been successful in reducing the incidence of sepsis in burn patients. A similar approach might be useful in the granulocytopenic cancer patient.

Method:

Cancer patients who are about to receive drug therapy which would be expected to produce leukopenia below 1000 WBC/mm^3 are entered on study. Patients are assigned at random, in double blind fashion, to receive either 20 cc of Pseudomonas polyvalent hyperimmune globulin (supplied by Parke Davis and Company) or 20 cc of commercial gamma globulin. Injections are repeated every two weeks until the patient's white blood cell count returns toward normal. Serum samples are collected to determine titer rises. Twice weekly cultures of stool and gingiva are obtained. Patients are examined regularly for evidence of infection.

Major Findings:

As of February 1, 1971, 17 patients had been entered into each arm of the protocol. A total of three Pseudomonas aeruginosa infections have been encountered. These numbers are too small as yet to make meaningful comparisons. No significant side reactions have been encountered. No appreciable titer rises have occurred.

Proposed Course: Continue investigation.

Significance to Cancer Research:

A significant reduction in the development of pseudomonas infections in this patient population would markedly reduce the overall morbidity and mortality presently occurring in cancer patients made granulocytopenic with chemotherapy.

Honors and Awards: None.

Publications: None.

Serial No. NCI 6266
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Carbenicillin-Gentamicin Therapy for Febrile, Granulocytopenic Cancer Patients

Previous Serial Number: None

Principal Investigator: Stephen Schimpff

Other Investigators: Viola M. Young and Arthur Serpick

Cooperating Units: None

Man Years

Total:	1/16
Professional:	1/16
Other:	0

Project Description:

Objectives:

Infection is a frequent and serious complication in the granulocytopenic patient with cancer. Bacteremia commonly occurs and usually leads to rapid demise. This study was designed to assess the efficacy of two antibiotics, given as initial empiric therapy, in the therapy of presumed sepsis in granulocytopenic cancer patients.

Major Findings:

Seventy-five patients were treated of which 48 had subsequent proof of infection. Of these 48 patients, 28 improved, 15 did not, and 5 could not be evaluated. Twenty-one patients had Pseudomonas aeruginosa infection; 80% improved. Results against infections due to other gram negative organisms were not as good. For this reason, the study has been discontinued and a new antibiotic combination has begun.

Proposed Course: Study completed December 31, 1970.

Significance to Cancer Research:

This antibiotic regimen has significantly reduced the mortality of Pseudomonas aeruginosa sepsis and, as such, has permitted prolongation of life

so that more adequate cancer chemotherapy could be used.

Honors and Awards: None

Presentations:

Empiric Therapy with Carbenicillin and Gentamicin for Febrile, Granulocytopenic Cancer Patients. Eastern Section of the American Federation for Clinical Research in New York, New York. December, 1970.

Publications:

Schimpff, S., Satterlee, W.,Young, V.M., and Serpick, A.: Empiric Therapy with Carbenicillin and Gentamicin for Febrile, Granulocytopenic Cancer Patients. New England Journal of Medicine. (in press)

Serial No. NCI 6267
1. BCRC
2. Medicine Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Keflin-Carbenicillin-Gentamicin as Empiric Therapy of Granulocytopenic Cancer Patients

Previous Serial Number: None

Principal Investigator: William Greene

Other Investigators: Stephen Schimpff, Viola M. Young, and Jerome Block

Cooperating Units: None

Man Years

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives:

The preceding study using carbenicillin-gentamicin for empiric therapy of presumed sepsis indicated the efficacy of that regimen in reducing the morbidity and mortality of Pseudomonas aeruginosa infection in granulocytopenic cancer patients. However, the poor success against other gram negative organisms, especially Klebsiella, indicated that gentamicin was not adequate for those infections (Klebsiella are usually resistant to carbenicillin but sensitive to gentamicin). The carbenicillin-gentamicin combination was retained because of its proven usefulness against Pseudomonas aeruginosa. Keflin has been added because it is bactericidal against Klebsiella and may prove to be synergistic with gentamicin against this organism. Furthermore, all of the other commonly encountered pathogens at this Center, except for the fungi, are sensitive to gentamicin. The addition of Keflin should strengthen the usefulness of the combination against gram positive organisms, notably Staphylococcus aureus. It is hoped that this combination, used at the first evidence of systemic infection, will be active and efficacious against the bacterial infections at this institution.

Major Findings: None as yet.

Proposed Course: Continue for approximately 18 months.

Significance to Cancer Research:

The major limiting factor in cancer chemotherapy is the high incidence of severe and often fatal infection. Any efficacious means of preventing or treating these infections should result in more effective drug therapy of cancer.

Honors and Awards: None

Publications: None

Serial No. NCI-6021
1. Baltimore Cancer Research Center
2. Neurosurgical Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Mechanism of Hydroxylation of Foreign Compounds.

Previous Serial Number: NCI-6021

Principal Investigator: Jordan L. Holtzman, M.D., Ph.D.

Other Investigators: Barry H. Rumack, M.D.

Cooperating Units: None

Man Years: (July 1, 1970 to June 30, 1971)

Total: 1.0
Professional: 1.0
Other: 0.0

Project Description:

Objectives:

The major pathway in the mammalian organism for the synthesis and metabolism of steroids and the transformation of lipid soluble drugs to polar metabolites, involves enzymatic reactions which require molecular oxygen and reduced nicotinamide-adenine dinucleotide phosphate (NADPH). Because of the known importance of these enzymes in the metabolism of drugs and steroids, as well as their postulated significance in the activation of many carcinogenic agents to form the proximal carcinogen, it is important to understand the mechanism of these reactions.

In 1968 and 1969, P. L. Gigon, T. E. Gram and J. R. Gillette observed that the rate of drug hydroxylations was stoichiometric with the rate of reduction of the substrate-cytochrome P-450 complex. In the fiscal year 1970 report, we noted that the inhibition of the overall ethylmorphine N-demethylase by D_2O correlated well with the inhibition of the ethylmorphine stimulation of the NADPH-cytochrome P-450 reductase activity lending further support to the suggested regulation of hydroxylation by the stimulatory process. Because of the importance of this reaction in drug metabolism, we have examined the kinetics of ethylmorphine stimulation of the reductase (ΔRed).

Methods Employed:

Standard biochemical techniques were employed.

Major Findings:

The double reciprocal plot of ΔRed vs ethylmorphine concentration indicates that in the male rat there are two activation sites, one with an affinity constant, KΔ, of 0.02-0.04 mM and a second one with a KΔ of 0.6-1.5mM. The composite of these two sites gives an apparent KΔ of 0.1 mM. The comparison of this latter value to the Km for the ethylmorphine N-demethylase, which in in these and previous studies has been shown to be 0.2 mM in the male rat, would indicate that the Km for the N-demethylase reaction in the male is determined by the composite of the two reductases. The type I binding constant for ethylmorphine, Ks, is 0.055 mM. It is possible that this site is closely related to the high affinity activation site.

The picture in the female rat is quite different. There is only a single activation site for ΔRed with a KΔ of 0.08 mM which compares quite nicely with the type I binding constant of 0.06 mM, but differs markedly from the Km for ethylmorphine N-demethylase, which is 0.6 mM. These results would suggest the existance of yet another site, the catalytic site which actually binds the substrate molecule to be hydroxylated, and which determines the Km in the female rat.

Significance to Bio.-med. Research and Program of the Institute:

These results confirm the importance of the activation site in the *in vitro* metabolism of drugs. Further the presence of a high affinity site in both sexes of the rat would suggest that the stimulation of NADPH-cytochrome P-450 reductase by ethylmorphine is an important process in determining the rate of hydroxylations *in vivo* as well as *in vitro*.

Proposed Course: None

Honors and Awards:

Dr. Holtzman received a travel grant from the Swedish Medical Research Council to attend the "International Symposium of Drug Metabolism" to be held June 2 to June 4, 1971 in Skokloster, Sweden. He will also present "The Kinetics of Ethylmorphine Stimulation of NADPH-Cytochrome P-450 Reductase in Hepatic Microsomes from Male and Female Rats" at this meeting.

Publications:

L. E. May and J. L. Holtzman: Mossbauer Spectroscopy of Lyophilized Rat Liver Microsomes. Biochem. Biophys. Res. Commun., 39: 296-300, 1970.

J. L. Holtzman and M. L. Carr: Inhibition of Hepatic Microsomal Mixed-Function Oxidases by D_2O. Life Sciences, 9: 1033-1038, 1970

J. L. Holtzman: The Effect of 4,4-Dideuteration of Nicotinamide-Adenine Dinucleotide Phosphate on Steroid Hydroxylation. J. Amer. Chem. Soc., 92: 6993-6994, 1970.

Serial No. NCI-6022
1. Baltimore Cancer Research Center
2. Neurosurgical Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Effect of Various Factors on the Hepatic Microsomal System and Associated Metabolic Pathways.

Previous Serial Number: NCI-6022

Principal Investigator: Jordan L. Holtzman, M.D., Ph.D.

Other Investigators: Barry H. Rumack, M.D.

Cooperating Units: None

Man Years: (July 1, 1970 to June 30, 1971)

Total: 0.5
Professional: 0.5
Other: 0.0

Project Description:

Objectives:

Numerous factors have been shown to markedly alter both the drug metabolizing enzymes of the hepatic endoplasmic reticulum and the chemical composition of this organelle. Among these factors are sex, age, disease state, dietary manipulations, and drug administration. Previous investigations in this and other laboratories have indicated that the increase observed in microsomal enzymatic activity, protein and heme on the administration of phenobarbital results from an increase in the synthesis of these constituents. On the other hand, the increase in microsomal phospholipid results primarily from the inhibition of phospholipid catabolism. Although these broad outlines of phenobarbital action have been well worked out, the very important details of the proximal mechanism of these effects remain to be elucidated. Phenobarbital could therefore act at several sites. Among them are: binding to some cytoplasmic factor to release the inhibition of a cytoplasmic repressor, direct stimulation of the genome, inhibit nuclear or microsomal nuclease activity, or enhance translation. In the present study we have examined the possible interaction of phenobarbital with the hepatic microsomes after *in vivo* administration in order to determine whether the effect of this drug could be directly on some component of the microsomes.

Methods Employed:

Standard biochemical techniques were employed.

Major Findings:

Freshly prepared hepatic microsomes from animals pretreated with phenobarbital (80 mg/kg/day x's 3 or 4) would usually exhibit the usual type I binding spectrum with ethylmorphine, while in otherpreparations or in aged preparations there was almost no binding spectrum in spite of good N-demethylase activity. The addition of albumin (0.5 mg/ml) to the sample side of the spectrophotometer without the presence of substrate led to the presence of the mirror image of a type I spectrum except that the peak was shifted to 412 nm rather that the expected 423 nm. When albumin was added to both sides and a low concentration (0.1 mM) of ethylmorphine was used, there appeared a normal type I spectrum, but when the concentration of ethylmorphine was increased to 2 mM, the trough than had a minimum at 412 nm. This would suggest the existance of two type I binding sites in the phenobarbital treated animals. Further, the spectral evidence would indicate the binding of the administered phenobarbital to the microsomes. And indeed when 2-C-14-phenobarbital was used for the induction, there proved to be 0.25 µg of phenobarbital found per mg protein which was equivalent to 0.556 nmoles of phenobarbital per nmole of cytochrome P-450. The former figure had a coefficient of variation of 27% while the latter had one of 3.1% suggesting that the phenobarbital directly bound to the cytochrome or rather to half of the cytochromes. Of the bound label, at least 70% would appear to be the parent compound.

Significance to Bio-med. Research and Program of the Institute:

These data lend further evidence that the inductive action of phenobarbital may be due to direct action on the microsomal membrane.

Proposed Course: None

Honors and Awards: None

Publications:

J. L. Holtzman, T. E. Gram, and J. R. Gillette: The Kinetics of 32-P Incorporation into the Phospholipids of Hepatic Rough and Smooth Microsomal Membranes of Male and Female Rats. Arch. Biochem. Biophys., 138: 199-207, 1970.

Serial No.: NCI-6024
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of the Neurosurgical Service With the Primary Interest in the Treatment of Brain Tumors at the BCRC, NCI

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Mark L. Rosenblum, M.D.
Kendall A. Smith, M.D.
Arden F. Reynolds, Jr., M.D. (USPHS)

Cooperating Units: Medical Service, BCRC, NCI
Department of Surgery, USPHS Hospital, Baltimore, Maryland

Man Years:

Total:	3.0
Professional:	3.0
Other:	0

Project Description:

Objectives - Major:

This ongoing project is designed to develop an effective research neurosurgical service to evaluate various modalities of treatment of intracranial neoplasm.

Objectives - Minor:

1. Continued development of the Brain Tumor Study Group. As Chairman, conduct and participate in meetings, visit various neurosurgical centers, assist in development of protocol and the evaluation of the data generated therefrom.

2. Supervision of repetitive ventricular perfusions with chemotherapeutic agents in conditioned monkeys at the A. D. Little Co. Boston, Mass.

Objectives - Minor (cont.)

3. Supervision and consultation with the puppy brain tumor oncology program for developing experimental model at the A. D. Little Co., Boston, Mass.

4. Continued investigation into the distribution, metabolism and cellular fractions of steroids in cat brain as a joint project with members of the National Heart Institute.

5. Drug distribution and pharmacologic-physiologic studies in elasmobranchs, particularly in reference to the blood-brain barrier and central nervous system.

Methods Employed:

Major Program:

Patients with intracranial neoplasm are referred to the BCRC, Section of Neurological Surgery, for evaluation and chemotherapy both from within the private community, as well as the PHS system. Primary unoperated upon CNS tumor is preferred, so that pharmacologic evaluation of chemotherapeutic agents, intracranial markers, etc., may be carried out as part of the operative procedure.

Frequent informal consultations and neurologic evaluation of BCRC patients are provided the medical staff to assist in the general medical program. A particular interest in CNS leukemia, as well as metastasis of other tumors, is pursued. CSF clearance of inert substances has been carried out.

General neurologic and neurosurgical consultation and operative assistance are given to the USPHS Hospital, Baltimore, Md., in the treatment of neurosurgical disease. Participation in general hospital practice and informal teaching by discussion or consultation have been pursued.

Major Findings:

Listed below are the data related to the patients cared for by The Section of Neurological Surgery, BCRC, NCI, from May 1970 through March 1971.

Major Findings (cont.):

	TOTAL	PHS	BCRC
CONSULTATIONS (formal)	63	39	24
OPERATIONS and PROCEDURES			
Craniotomy	8	1	7
Laminectomy	2	2	0
Other	5	4	1
ADMISSIONS	115	3	112

Admissions to the Section of Neurological Surgery have increased by 10%. This reflects both detailed follow-up of patients on continuous treatment, as well as new patient referrals.

Twenty-five patients have been placed on CCNU protocol (130-150 mg/m^2/day q. 8 weeks). About half of these patients have shown some remission referable to their therapy. The duration of remission is variable, dependent upon stage and location of disease. Toxicity with this therapy has been minimal, and only one patient has required platelet transfusion.

Epipodophyllotoxin-PTG (NSC 122819) has been designed in a protocol for weekly treatment, and patients who become refractory to the nitrosoureas or have severe hematologic depression, will be treated with this drug (100 mg/m^2 weekly x 8).

The Brain Tumor Study Group is more than half way through a well-controlled prospective, randomized study of BCNU in comparison with BCNU and Irradiation, Irradiation Alone, and Continued Best Conventional Care (the control group). Preliminary analysis of the data tends to indicate that there may be some differences between the various treatment groups.

CSF space perfusions at Arthur D. Little using 8-Azaguanine have indicated that 1.0, 0.5, and 0.25 mg/ml of 8-Azaguanine is above the threshold dose and produces neurologic and behavioral impairment. Further perfusions to delineate a maximally tolerated dose without obvious signs are being pursued.

The Rous sarcoma induced puppy brain tumor has been carried forward in serial transplant and has been shown to be responsive to treatment with both BCNU and CCNU. Further studies with cytoxan epipodophyllotoxin and several other drugs are planned.

Publications and Presentations:

Publications:

Fontana, J. A., Walker, M. D., Casper, A. G. T., Meret, S. and Henkin, R. I.: Sequential Subcellular Localization of Cortisol in Cat Brain. Endocrinology 86: 1469-1471, 1970.

Hurwitz, B. S., Sutherland, J. C. and Walker, M.D.: Central Nervous System Chloromas Preceding Acute Leukemia by One Year. J. Neurology 20: 771-776, 1970.

Walker, M. D. and Hurwitz, B. S.: BCNU, 1-3-Bis-(2-Chloroethyl)-1-Nitrosourea (NSC 409962) in the Treatment of Malignant Brain Tumor. A Preliminary Report. Cancer Chemother. Rep. 54,4: 263-271, 1970.

Walker, M. D., Henkin, R. I., Harlan, A. B. and Casper, A.G.T.: The Distribution of Tritiated Cortisol in Blood, Brain, CSF and Other Tissues of the Cat. Endocrinology 88: 224-232, 1971.

Walker, M. D., Rumack, B. H. and Rosenblum, M. L.: Malignant Central Nervous System Tumor and Other Neoplasms in a Large Family. Neurology 21,4: 1971.

Hansen, H. H., Selawry, O. S., Muggia, F. M., and Walker, M.D.: Clinical Studies with 1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea (CCNU) (NSC 79037). Cancer Res. 31,3: 223-227, 1971.

Article in Bulletin:

Walker, M. D. and Henkin, R. I.: Blood-brain Barrier Considerations of Corticosteroids. Psychopharmacology Bulletin. (In press)

Presentations:

Mount Sinai Hospital, New York, N. Y. "Recent Progress in Chemotherapy of Brain Tumors with BCNU and Other Agents."

Brooklyn Neurological Society. "Chemotherapy of Brain Tumors."

American Physical Therapy Association of Maryland. "Chemotherapy of Brain Tumors and Its Implications for Rehabilitation."

Lahey Clinic Foundation Alumni Meeting. "Recent Methods of Chemotherapy."

Congress of Neurological Surgeons Meeting. "Evaluation of Head and Neck Infusion."

Presentations (cont.):

American College of Neuropsychopharmacology. "Blood-brain Barrier Considerations of Corticosteroids."

American Association for Cancer Research. "The Treatment of Brain Tumor With 1-(2-Chloroethyl)-3-Cyclohexyl-1-Nitrosourea (CCNU)."

Serial No.: NCI-6202
1. Baltimore Cancer Research Center
2. Section of Neurological Suragery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Murine Intracerebral Melanoma Study

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Donald M. Bowie, B.S.

Cooperating Units: None

Man Years:

Total:	.9
Professional:	.9
Other:	0

Project Description:

Objectives:

The major objectives of this project are to develop an additional murine intracerebral tumor, which also grows subcutaneously, for evaluation of the pharmacology, tumor kinetics and efficacy of chemotherapeutic agents. In addition, the interrelationship of one drug upon the metabolism efficacy and toxicity of another is being studied (so-called drug-drug interaction.)

Methods Employed:

B-16 melanoma of the Jackson Laboratory, Bar Harbor, Maine, has been employed for serial transplant as described previously. Treatment schedules with CCNU and Methyl CCNU at varying dose levels have been employed.

Major Findings:

Single dose therapy with CCNU or Methyl CCNU appears to increase life span by 25-50%. However, there appear to be no cures.

Proposed Course:

Further studies with this model will be pursued utilizing these drugs in combination, as well as epipodophyllotoxin. Drug-drug interactions with these substances will be evaluated.

Serial No.: NCI-6203
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of Drug Distribution in Patients and Human Tumor

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: V. T. Oliverio, Ph.D., V. T. DeVita, M. D. M. L. Rosenblum, M.D., K. A. Smith, M. D. and A. F. Reynolds, Jr., M.D. (USPHS) and Donald M. Bowie, B.S.

Cooperating Units: Medical Service, NCI, NCI-LCP.

Man Years:

Total:	.6
Professional:	.6
Other:	0

Project Description:

Objectives:

This is a continuation of a project whose major objectives are to evaluate the pharmacologic absorption, distribution, excretion of antineoplastic agents in patients. This is done, in particular, in coordination with surgical therapy, so as to obtain tissue samples.

Methods Employed:

Patients undergoing surgery for tumor, who are receiving chemotherapy, receive C^{14} CCNU with the radio-label in one of three positions. Samples of blood, urine, cerebral spinal fluid, muscle, brain, and tumor are obtained for analysis of content of drug, as well as metabolites.

Major Findings:

All three labels of CCNU C^{14} (carbonyl, cyclohexyl, and chloroethyl) have been evaluated after administration by mouth. Radioactivity appears in plasma promptly, but less than 25% of this

Major Findings (cont.):

is lipid extractable drug. Similarly, significant amounts of C^{14} are found in CSF, brain, muscle, and tumor, but little of this is parent compound. Since the drug produces marked hematopoietic toxicity, has an extremely prolonged plasma radioactive half-life with over three-quarters of the administered dose being recovered in the urine and appears promptly in tissues, it is of importance to determine which part of the compound is active.

Proposed Course:

This project is being completed and prepared for publication.

Serial No.: NCI-6204
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Toxicity of Intracerebral Chemotherapy in Monkeys

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Dan Dalgard, D.V.M.

Cooperating Units: Hazelton Laboratories, Falls Church, Va.

Man Years:

Total:	.9
Professional:	.9
Other:	0

Project Description:

Objectives:

In this ongoing project, a relatively standardized technique for evaluating intrathecal chemotherapy in monkeys has been developed. With the increase in meningeal leukemia and the interest in neoplasms within the central nervous system, the use of intrathecal chemotherapy is expected to increase.

Methods Employed:

Acute and chronic intracisternal installation of appropriate drugs and evaluation of activity indices, hematologic parameters, and cerebral spinal fluid are made.

Major Findings:

Thiotepa, an alkylating agent, is in the process of being evaluated for possible intrathecal use. Final pathology reports are not yet prepared.

Proposed Course:

With the increased interest in the possible use of intrathecal drug, a separate program will be started in order to hasten this activity.

Serial No.: NCI-6233
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Determination of Human CSF Clearance

Previous Serial Number: SAME

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Kendall A. Smith, M.D.

Cooperating Units: Medical Service, BCRC, NCI

Man Years:

Total:	.2
Professional:	.2
Other:	0

Project Description:

Objectives:

This project is designed to determine the rate of clearance of drugs placed in the lumbar thecal space in patients receiving chemotherapy for neoplastic disease.

Methods Employed:

Patients harboring neoplasms receiving lumbar puncture for diagnostic evaluation or installation of chemotherapy receive tracer doses of ^{14}C Inulin during the procedure. Because of complete glomerular filtration of Inulin, any Inulin which appears in the plasma is promptly cleared and may be found in the urine. Therefore, urine is analyzed for Inulin content and the clearance rate calculated.

Major Findings:

CSF clearance of Inulin placed in the lumbar space has a half-life in the order of 15 hours. Plasma clearance of the same substance is approximately 3 hours; hence, CSF half-life clearance is approximately one-half day.

Major Findings (cont.):

Proposed Course:

Pure radio-labeled pyrogen-free methotrexate has been obtained and will be utilized in comparison with Inulin clearance.

Serial No.: NCI-6268
1. Baltimore Cancer Research Center
2. Section of Neurological Surgery
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Radio-labeled Distribution of Methyl CCNU In Dogs

Previous Serial Number: NONE

Principal Investigator: Michael D. Walker, M.D.

Other Investigators: Arden F. Reynolds, Jr., M.D.
Mark L. Rosenblum, M.D.
Donald M. Bowie, B. S.

Cooperating Units: None

Man Years:

Total:	.6
Professional:	.6
Other:	0

Project Description:

Objectives:

This project is designed to determine the pharmacologic characteristics of methyl CCNU in the dog as preparation for similar determinations in humans.

Methods Employed:

Following intravenous or intragastric administration of C^{14} Me-CCNU, serial blood, CSF, urine, bile, muscle and brain samples have been obtained from 4 minutes up to 24 hours. Direct sample counting, as well as extraction and chromatography, have been carried out. The development of an adequate (fat emulsion) vehicle for the carrying of highly lipid and soluble substances has been evaluated.

Major Findings:

A suspension of fat emulsion is able to solubilize methyl CCNU and carry it in aqueous solution. The drug possesses the characteristics of a lipid soluble substance and following intravenous administration, enters brain and muscle promptly. Not all recoverable drug, however, is parent compound. Over half the

Major Findings (cont.):

administrated radio-label appears in bile and urine. Following itragastric administration, there is no detectable methyl CCNU in plasma, although radioactivity is present.

Proposed Course:

This project will continue with evaluation of two other labels of methyl CCNU in preparation for human pharmacologic evaluation.

Serial No: NCI-6043
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Automated Monitoring of Drug Therapy

Previous Serial Number: Same

Principal Investigators: Clarence L. Fortner, Gerald Goldman, and Jerome Block

Other Investigators: Richard Hsieh, Barbara Murray, James Cradock, and Stephen Schimpff

Cooperating Units: Division of Health Services Research, Baltimore USPHS Hospital; Program Analysis Branch, Chemotherapy, NCI, Bethesda, Maryland

Man Years:

Total: 4
Professional: 2
Other: 2

Project Description:

Objectives:

To provide inexpensive, efficient and rapid method of collecting data concerning the clinical trials of experimental drugs.

Methods Employed:

An automated method of monitoring drug therapy was designed and implemented.

An inexpensive pen and paper system of processing drug information provided accurate, periodic summaries of patient's drug therapy. A medication for each of a patient's drugs is marked at the time of drug administration. Drug and patient identifiers are typed on the card and this data is read weekly by optical character recognition. The data is collated and a weekly summary is printed listing total dose, frequency of administration or omission, and errors in administration of each drug. A cumulative report printed weekly lists cumulative doses for all drugs the patient has received.

The physician writes his order directly on the medication card that produces

Methods Employed (cont'd)

four copies: the medicine card copy, the pharmacy copy, and copies for medical records and automated analysis. A clerk types the drug name, dosage information, patient name and record number and date drug was initiated. Most intravenous medications can be indicated by "check-off" boxes and each drug administration or drug omission is "checked-off" by the nurse.

Major Findings:

The medication card fulfills all the requirements of drug ordering, administration and recording, in addition to providing machine-readable data for computer input. The summaries are checked by physicians, pharmacists and nurses and become a permanent record of drug therapy. This system eliminates the transcription operations usually associated with preparation of input for computers, and provides a data pool for clinical studies, analyzing drug effect, toxicity, and drug interaction. This system has reduced errors in drug administration and has increased staff awareness of the problems inherent in quality drug administration.

Significance to Bio-medical Research:

This system, as outlined, has provided a more comprehensive and efficient record of cancer chemotherapy drug trials with more rapid and analytical results.

Honors and Awards: None

Publications: None

Serial No: NCI-6236
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Role of the Pharmacist in Obtaining a Patient's Drug and Allergy History

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: J. Cradock, and G. Whitfield

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1 1/3
Professional: 1 1/3
Other: 0

Project Description:

Objectives:

To evaluate the feasibility of having a pharmacist interview all patients admitted to the Baltimore Cancer Research Center for the purpose of obtaining a medication and allergy history.

Methods Employed:

The pharmacist questions each patient admitted to the Medicine and Neurosurgical Services, BCRC, concerning current and past medication usage and history of allergy.

A written record of this interview is placed in the history section of the patient's chart.

Major Findings:

The pharmacist has proved to be an effective interviewer in obtaining drug and allergy history information. In most instances the information obtained by the pharmacist is quite similar to that recorded by the physician. However, the pharmacist is able to more readily identify drug products, and he obtains more information about the patiert's use of non-prescription medication than the physician records.

Significance to Bio-medical Research:

More complete histories of medication usage are provided which may result in improved care of patients.

Proposed Course:

The present study will be continued.

Honors and Awards: None

Publications: None

Serial No: NCI-6237
1. BCRC
2. Pt. Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of a Patient Care Pharmacy Service

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: J. Cradock, G. Whitfield

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1 1/3
Professional: 1 1/3
Other: 0

Project Description:

Objective:

To determine the pharmacist's role in the delivery of health care in a patient care area.

Methods Employed:

The pharmacist makes patient rounds with medical and nursing staffs. He reviews drug orders and maintains drug profiles on all patients. The profiles are used in the determination of drug-drug interactions, drug-dietary interactions, and drugs that modify laboratory results.

Drug information is provided for the medical and nursing staff by several means; providing verbal or written answers to questions, presenting lectures on pharmacology, and contributing to a drug newsletter.

Major Findings:

The pharmacist has been readily accepted in the patient care area and has functioned well. Several points concerning drugs have been brought to the attention of members of the patient care team by the pharmacist.

Significance to Biomedical Research:

These contributions have increased the quality of patient care at the Baltimore Cancer Research Center.

Proposed Course:

Present studies will be continued and additional methods to improve patient care, such as unit dose drug distribution, will be developed.

Honors and Awards: None

Publications: None

Serial No: NCI-6238
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Preparation of Parenteral Drugs by a Patient Care Pharmacy Service

Previous Serial Number: Same

Principal Investigator: Clarence L. Fortner

Other Investigators: J. Cradock, and G. Whitfield

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1 1/3
Professional: 1 1/3
Other: 0

Project Description:

Objectives:

To improve patient care and to relieve the medical and nursing staffs of some pharmacy-related duties by making the pharmacist responsible for the preparation of parenteral medication. To provide more accurate information regarding the disposition of investigational drugs.

Methods Employed:

All products are aseptically prepared in a laminar air flow environment and dispensed in a clearly labelled, ready to use form. Control procedures for checking the composition of the products prepared and for monitoring bacterial and particulate matter contamination of these preparations have been instituted. Pertinent information regarding the drug dispensed is recorded on specially designed forms that are filed according to the patient's name. This information is also recorded by drug lot for investigational drugs.

Major Findings:

1. Quality products for parenteral administration are produced.

2. Readily retrievable information concerning the parenteral chemotherapy

Major Findings Continued:

received by a particular patient, and the disposition of each lot of drug is provided.

Significance to Biomedical Research:

Physicians and nurses are relieved of some pharmacy-related tasks and are able to devote more of their time to patient care. Accurate records of the disposition of parenteral cancer chemotherapeutic agents are provided.

Honors and Awards: None

Publications:

1. Menzie, J., Cradock, J., and Fortner, C.L.: Development of a Unit Dose System for the Handling of Parenteral Investigational Cancer Chemotherapeutic Agents. In press, Amer. J. Hosp. Pharm.

2. Fortner, C.L., Foley, T., and Cradock, J.: Reduction in serum CO_2 content during hyperalimentation therapy. Amer. J. Hosp. Pharm. 28: 121-122, 1971

Serial No: NCI-6269
1. BCRC
2. Patient Care Pharmacy
3. Baltimore, Md.

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Development of a Unit Dose Service

Previous Serial Number:

Principal Investigator: Clarence L. Fortner

Other Investigators: J. Cradock, and G. Whitfield

Cooperating Units: Medicine and Neurosurgical Service, BCRC

Man Years:

Total: 1 1/2
Professional: 1 1/2
Other: 0

Project Description:

Objective:

To improve patient care and to relieve the nursing staff of some pharmacy-related duties by making the pharmacist responsible for the preparation of drugs in a unit of use form.

Methods Employed:

A unit dose of medicine is a single dose of a drug packaged for an individual patient. This single dose package is labelled with the name, strength, and control number of the drug and it is ready to be administered to the patient. The drugs are ordered by the physician and they are placed in an individual patient's drawer of a medication cart by the pharmacist. This cart is designed so that there is a single drawer for each patient's drugs. The medication cart with the drugs ready to administer is transported to the nurse.

Major Findings:

1. Nurses are relieved of many non-nursing duties such as, requisitioning drugs, storing drugs, preparing medications for administration, etc. thereby increasing the nurse's time for patient care.
2. Reduces medication errors because of the checks by the pharmacist and nurse built into the system.

Major Findings (cont'd)

3. Reduces the drug inventory on each nursing unit.

4. Delivers better drug control which reduces drug loss.

Significance to Bio-medical Research:

Nurses are relieved of some pharmacy related tasks and are able to devote more of their time to patient care. This system has increased the accuracy of drug records and drug administration.

Honors and Awards: None

Publications: None

Serial No. NCI 6023
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Radiation Therapy Service

Principal Investigator: Kirkland C. Brace, M.D.

Other Investigators: James D. Easley, M.D.

Cooperating Unit: Medicine Service, Baltimore Cancer Research Center and U.S. Public Health Service Hospital, Baltimore, Maryland

Man Years: (July 1, 1970 - June 30, 1971)

Total: 3-1/2
Professional: 2
Others: 1-1/2

Project Description:

Objectives:

To provide the best possible radiation therapy for the patients at this Institution. The purpose of this facility is to investigate the use of combinations of radiotherapy and chemotherapy in the treatment of malignant lymphomas and other tumors. About 50% of the activities of this Department are devoted to the treatment of the cancer patients from the general hospital population.

Methods Employed:

All modes of radiation therapy available here were utilized for the treatment of all types of cancer. This equipment consists of a 100 Kv Superficial therapy machine, a 285 Kv Orthovoltage machine and a Cobalt therapy unit. It is also equipped with a supply of Cesium, Radium and Strontium sources for interstitial and intracavitary radiation therapy.

Major Findings:

The Radiation Therapy Service has been in operation for three years at this Institution. During the calendar year ending March 31, 1971, 197 patients were seen in consultation from the other services. Of these 115 were referred from the Medicine Service of the Baltimore Cancer Research Center and the balance of 82 from the general hospital. A total of 107 patients were

treated for the Tumor Service and the remainder of 67 for the Hospital. The breakdown of the treatments follow (April 1, 1970 - March 31, 1971):

	Tumor Service	Hospital Services
Superficial Therapy	11	14
Orthovoltage Therapy	24	1
Cobalt Therapy	2677	1255
Intracavitary or Interstital Radium & Cesium Applications	0	1
Strontium-90 Applications	0	19

Significance:

There has been no decrease in the patient load in spite of a general reduction in the number of research beds available to the Baltimore Cancer Research Center. The loss of these patients has been made up by an increasing number of patients referred in from the general hospital. The U.S. Public Health Service Hospital in Baltimore has become a cancer treatment center. Our principle interest is in the development of new treatments for patients with lymphomas.

Course:

The treatment of cancer patients will continue on both the Tumor Service and the general hospital service. The loss of our Physicist continues to hamper the general operation and reduce our ability to provide specialized forms of treatment. Our Cobalt source has been replaced and should provide adequate output for the next three years. We hope that at some future time funds will be available to provide us with a modern Linear Accelerator to improve our physical facilities.

Serial No. NCI 6207
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Radiological Physics

Principal Investigator: Doris Husak Johns, M.S.

Man Years: (July 1, 1970 through June 30, 1970)

Total: 2 months
Professional: 2 months
Other: 0

Project Description:

Objectives:

To provide the principles of Radiological Physics to the treatment of patients, under the supervision of a Radiotherapist.

To provide non-patient service irradiations.

To provide a Radiation Safety Program for the Pharmacology Laboratories and Radiotherapy Department.

Methods Employed:

The three Radiation Therapy machines are calibrated periodically. Treatment plans are provided on request of the Radiotherapist. Calibrations are kept on all sealed radionuclide sources and doseage calculations are provided on request of the Radiotherapist.

The program was terminated on September 1, 1970 when the Physicist resigned and was not replaced.

Invitro and animal irradiation was provided to investigators in the Pharmacology Laboratory for a total of 102 service irradiations. Irradiation service has been provided for Johns Hopkins, Laboratory of Biology for 237 rabbits and guinea pigs.

A Radiation Safety Program is maintained for the Pharmacology Laboratories in which radionuclides are kept or used. These laboratories are surveyed periodically for radionuclide contamination. The Radiation Therapy facilities (Cobalt-60 Teletherapy unit and various sealed sources of Radium, Cesium-137 and Strontium-90) are surveyed according to AEC Regulations.

Major Findings:

There has been no detectable leakage of any Radiotherapy sources, nor any significant amount of contamination in any Pharmacology Laboratory.

Proposed Course:

It is anticipated a new Physicist will be hired during the next few months to reactive the program and place our surveys on a current basis.

Serial No: NCI -6270
1. Baltimore Cancer Research Center
2. Radiation Therapy Service
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Combination Chemotherapy and Radiotherapy in the Treatment of Hodgkin's Disease

Principal Investigator: Kirkland C. Brace,M.D., James D. Easley,M.D.

Other Investigators: Irvin Zarembok, M.D.

Cooperating Units: Medicine Service, Baltimore Cancer Research Center and U.S. Public Health Service Hospital, Baltimore, Maryland

Man Years:

Total: 1-1/2
Professional: 1-1/2
Others: 0

Project Description:

Objectives:

To study the use of a combination of MOPP (Mustard, Vincristine, Prednisone and Procarbazine) and radiotherapy in the treatment of Hodgkin's Disease.

Methods Employed:

The Stage I and II Hodgkin's Disease patients are randomly assigned to three treatment groups:

1. Radiation therapy to the involved node-bearing areas plus the adjacent areas.

2. Three courses of MOPP followed by radiation to the involved node-bearing areas.

3. Radiation to the involved area plus three courses of MOPP.

Major Findings:

There has now been two years of follow-up on twenty-one patients in this study. Two of the seven patients in the first group have relapsed in unirradiated areas. Five of seven of the second group have relapsed in unirradiated areas and none have recurred in the third group. It is already clear that three courses of

MOPP is not sufficient to control even the clinically undetectable disease when it is given prior to the radiation. There is suggestion that the chemotherapy delivered after the radiation may be useful in the control of the disease.

Significance:

If this data can be made to be significant, it would appear that it may be possible to treat Hodgkin's Disease with very limited irradiation to the detectable tumor areas followed by a short course of chemotherapy.

Course:

We plan to continue this study and expand it to include the Stage III disease during the next few years.

SUMMARY REPORT

LABORATORY OF PHARMACOLOGY

Continued work with daunomycin in man and in animals has clarified further the drug's metabolism. Several metabolites have been demonstrated with a proposed enzymic schema for daunomycin breakdown resulting in an aglycone and in a reduced moiety with the amino sugar intact which has at least as great activity in vitro against L_{1210} leukemia as the parent drug. Clinical studies of daunomycin drug metabolism during chemotherapy of leukemia are in progress in an effort to establish potential mechanisms of drug sensitivity and resistance.

Early work on hormonal-steroid-mediated effects on rat prostatic tissue enzyme levels have shown biochemical changes which may be important in regeneration of cellular proliferation. Plans are now to extend these studies to the hyperplastic gland.

Additional studies with the isolation and purification of specific bacterial nucleases for synthetic and naturally occurring nucleic acids has resulted in enzymic "reagents" for Poly A, Poly I, Poly U, and Poly C cleavage. These enzymes could prove useful for elucidating sequential structure of nucleic acids of normal and tumor nucleic acids. A study of polyamine metabolism and related enzymes has been initiated in embryonic, normal proliferating, and tumorous tissue. S-adenosyl methionine decarboxylase may be a key and rate limiting enzyme in such systems and, if so, opens the way for development of specific chemical inhibitory substances.

Work with several animal and tumor models is in progress to establish systems of allogeneic tolerance and evaluating host response to minor tumor histocompatibility differences. In vitro cell-cell interactions have been increasingly useful in evaluating these systems in the mouse and are now being used during the course of pilot immunotherapeutic programs in human acute leukemia. These latter studies were made feasible by perfecting these in vitro techniques and employing frozen and preserved normal and leukemic lymphocytes.

Electronmicroscopic and immunofluorescent studies have demonstrated evidence in human kidneys for possible viral antigen-antibody complexes. These have been demonstrated only rarely and in only a few patients with acute leukemia. Similar techniques in human acute myelocytic leukemic leukocytes have shown high organized cell-cell attachment complexes which have not previously been described, but appear to be associated with particulate matter ingestion.

Serial No. NCI-6011
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of Free Fatty Acids on Cation-stimulated ATPase

Previous Serial Number: NCI-6011

Principal Investigator: Khalil Ahmed

Other Investigators: B. S. Thomas

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971)

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives:

Cation-stimulated ATPase is now generally recognized as the enzyme system involved in the transport of Na^+ and K^+ across cell membranes. Therefore action of agents such as fatty acids which occur *in vivo* is of considerable importance. Several metabolic effects of fatty acids have been reported in literature with the hypothesis that these agents might have a regulatory role in cellular machinery. Further, free fatty acids have been shown by Shimkin et al to induce sarcomas at the site of injections in rats.

Methods Employed:

We have developed methods for the isolation of the $Na^+ + K^+$-stimulated ATPase and have reported in detail on the kinetics of interaction of Na^+ and K^+ with this enzyme system. These procedures have been used in the present investigation. Initial rates are measured in the presence and absence of fatty acids.

Major Findings:

Long chain fatty acids inhibit the cation-stimulated ATPase at very low concentrations *in vitro*. According to our kinetic analysis, the inhibition is freely reversible on removing the fatty acids from the enzyme. Unsaturated fatty acids are more potent inhibitors than their saturated congeners. The primary

site of action of myristate (studied as a representative fatty acid, and which is the most effective inhibitor among the long chain acids), appears to be on the K^+-stimulation of the enzyme system. This is further confirmed by our observation that myristate is a competitive inhibitor of the K^+-stimulated p-nitrophenylphosphatase, an enzyme activity originally described by us in 1962, and which is believed to be part of the K^+-stimulated step in the overall reaction of the ATPase system. The site of action of myristate appears to be distinct from those of the other commonly used inhibitors of transport ATPase, i.e., ouabain or oligomycin. Presumably fatty acids attach to the enzyme system at at site which overlap K^+ site and cause a conformational change which manifests itself as a competitive inhibition of the K^+-stimulated enzyme activity.

Significance to Bio-med. Research and Program of the Institute:

The report by Shimkin et al that injections of fatty acids produce sarcomas did not discuss the possibilities of the mode by which such a change was induced. Whereas, it is well-known that fatty acids inhibit numerous types of metabolic activities, eg., inhibition of oxisative phosphorylation and certain glycolytic enzymes, it should be borne in mind that maintenance of cellular ionic balance (in which the cation-stimulated ATPase is believed to be involved) is of a fundamental importance to the viability of whole cells and cell organelles. Thus our observations on the effects of fatty acids on transport ATPase provide a possible mode by which these agents might induce pathological phenomena, eg., degenerative changes as a consequence of excessive fat accumulation, aggregation of platelets by fatty acid, and induction of swelling in cells and organelles.

Proposed Course:

We hope to extend the study of action of fatty acids on other parameters of the transport enzyme and to further identify the mode of their action.

Honors and Awards: None

Publications:

Ahmed, K and Thomas, B.S.: The Effects of Long Chain Fatty Acids on Sodium-plus-Potassium Ion-Stimulated ATPase of Rat Brain. J. Biol. Chem., 246: 103, 1971.

Serial No. NCI-6012
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pharmacology of Anthracycline Antibiotics: Biochemical Pharmacology

Previous Serial Number: 6012

Principal Investigator: Nicholas R. Bachur

Other Investigators: Malcolm Gee
James Cradock

Cooperating Units: Pharmacy Service, USPHS Hospital, Baltimore, Maryland
Drug Development Branch, NCI, NIH
Laboratory of Chemistry, National Heart & Lung Institute

Man Years (July 1, 1970 to June 30, 1971)

Total:	1.5
Professional:	0.5
Other:	1.0

Project Description:

Objectives:

Since our previous descriptions of the metabolism of daunorubicin by tissue slices and homogenates of tissues, we have continued the study of the biochemical pharmacology of daunorubicin. From data in our early studies, we were aware that the metabolic pathways for daunorubicin were incompletely understood.

Major Findings:

Once we had shown that daunorubicin was metabolized by the soluble fraction from many tissues, we were able to design experiments to elucidate the metabolic steps and factors necessary for the metabolism of daunorubicin. As a result, we find that daunorubicin is reduced by an enzyme we have named daunorubicin reductase which is found as a soluble enzyme in every animal tissue we have investigated, including red blood cells. This enzyme apparently requires triphosphopyridine nucleotide as a cofactor. A second enzyme is found in liver microsomal fraction and in the sedimentable portions of several other tissues. This enzyme catalyses the hydrolysis of the glycosidic bond between

anthracycline nucleus of daunorubicin and the amino sugar, daunosamine. This enzyme also requires TPNH, apparently, and does not require oxygen. Most importantly we have observed that daunorubicin is metabolized by a constitutive enzyme unlike most other drugs.

The structure of the metabolites involved in daunorubicin metabolism were resolved through the use of ultraviolet visible spectroscopy, fluorescence spectroscopy, infrared spectroscopy, chemical derivatization, thin layer chromatography, mass spectroscopy. The major metabolic product which we have previously called D2 we now have named daunorubicinol, since it is the 13 hydroxy derivative of daunorubicin.

Significance to Bio-medical Research and Program of the Institute

Since the use of the cancer chemotherapeutic antibiotic daunorubicinol has grown significantly since the original clinical report by Tan et al, we feel it is increasingly important to understand as much as possible concerning the biochemical pharmacology of this drug so that it may be used judiciously and with some scientific basis. The findings that daunorubicin is metabolized by constitutive enzymes in situ, we feel, is a major step in understanding and correlating some of its pharmacologic actions.

Proposed Course:

To continue studies on the interrelationships of daunorubicin reductase and the pharmacologic properties of daunorubicin. We also intend to study adriamycin, another anthracycline antibiotic, in this system.

Honors and Awards: None

Publications:

Bachur, N. R. and Gee, M.: Daunorubicin Metabolism by Rat Tissue Preparations. J. Pharmacol. Exper. Therapu., in press.

Bachur, N.R.: Daunorubicinol, a Major Metabolite of Daunorubicin: Isolation from Human Urine and Enzymatic Reactions. J. Pharmacol. Exper. Therapu., in press.

Serial No. NCI-6014
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Cellular Control Mechanism Affecting Cell Growth and Differentiation.

Previous Serial Number: NCI 6014

Principal Investigator: Nicholas R. Bachur

Other Investigators: Marta Kaber

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971)

Total:	1.2
Professional:	0.2
Other:	1.0

Project Description:

Objectives:

Numerous studies have been carried out to elucidate cellular control that occur at the transcription and translative levels of the cellular replicative process. We have undertaken the study of a number of control points in cellular metabolism at the level of intermediary metabolism. Of interest has been the branch point for synthesis of a substrate which is utilized by both the urea cycle and in pyrmidine synthesis. This common substrate, carbamyl phosphate, is synthesized by the enzyme carbamyl phosphate synthetase, an enzyme which is under several metabolic controls. Although it is somewhat unclear, it appears that carbamyl phosphate is synthesized by two distinct enzymes in liver cells as well as in proliferating cells. We have undertaken a project to purify and characterize the carbamyl phosphate synthetase from normal and malignant tissues in an effort to assess the possibility that this point may be a convenient point for chemical intervention in the cellular metabolic process.

We have undertaken a study to investigate the control exerted on nonproliferating lymphocytes by agents which cause blastogenesis. Our studies have been aimed primarily at investigating alterations in cellular membrane processes. These are being carried out in order to determine differences between those lymphocytes which have a lower response to blastogenic stimuli and normal

lymphocytes. Earlier studies on human dihydrofolate reductase were gradually terminated.

Major Findings:

A method has been developed by which we are able to conveniently measure carbamyl phosphate synthetase by the incorporation of radiolabelled carbon dioxide into citrulline. The level of metabolic activity at this step is greatly modulated by the nutritional state of the animals, as has been shown in both mice and rats. On the other hand, carbamyl phosphate synthetase II which utilized glutamine is not affected by the nutritional parameters.

Significance to Bio-med. Research and Program of the Institute

By localizing points for critical control of metabolic pathways, we may find suitable approaches for the introduction of chemotherapeutic agents for controlling cellular growth and more differentiation.

Proposed Course:

To continue studies on carbamyl phosphate synthetase and lymphocyte blastogenesis. Our studies with dihydrofolate reductase will be terminated.

Honors and Awards: None

Publications: Jarabak, J. and Bachur, N.R.: A soluble dihydrofolate reductase from human placenta: Purification and properties. Archives Biochem. and Biophys. 142: 417-425, 1971.

Serial No. NCI-6016
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pharmacology of Cancer Chemotherapeutic Agents: Pharmacodynamics

Previous Serial Number: 6016

Principal Investigator: W. Delano Meriwether

Other Investigators: Nicholas R. Bachur
Richard Adamson

Cooperating Units: Laboratory of Chemical Pharmacology, NCI, NIH

Man Years (July 1, 1970 to June 30, 1971)

Total:	1.3
Professional:	1.3
Other:	0

Objectives:

To investigate the pharmacodynamics of cancer chemotherapeutic agents: I. The anthracycline antibiotics.

In earlier investigations, the mechanism of action of daunorubicin has been sited as being an inhibition of both RNA synthesis and/or DNA synthesis. Since the exact mechanism is unclear, we felt this problem warrented further investigation, especially in the light of our recent findings concerning the metabolism of daunorubicin. In addition, we have an interest in the pharmacologic action of a number of metabolites of daunorubicin or chemical derivatives of daunorubicin. These have been prepared by enzymatic conversion of daunorubicin to its metabolites and the chemical derivatives have been obtained from the Drug Development Branch.

Major Findings:

In an *in vitro* system consisting of L1210 tumor cells grown in spinner flask tissue culture we find that the inhibition of incorporation of tritiated thymidine and C14 uridine into DNA and RNA, respectively, is inhibited about equally by daunorubicin. Adriamycin also inhibits the incorporation of these precursors into DNA and RNA about equally. Interestingly, equimolar concentrations of daunorubicin and adriamycin have differing levels of inhibition.

We find that daunorubicin is a more effective inhibitor than is adriamycin. At levels approximating 2 micromolar, daunorubicin is approximately twice as effective as adriamycin. At 10 micromolar, it is approximately 25 per cent more effective. These findings are in contrast to what has been observed both in the intact animal or in clinical studies where adriamycin has been shown to be more effective for both producing clinical response and in producing toxicity than daunorubicin. Therefore, we feel that the effects that we observed in an in vitro situation must in some way be modulated to account for the observations in vivo. Parallel studies on the pharmacokinetics of these agents both in vivo and in isolated cell preparations are in progress and are expected to explain the interesting differences.

Studies with daunorubicinol, the predominant daunorubicin metabolite, also indicate a difference of inhibitory effect depending on the system used for the assay. We have also initiated a new series of studies concerning the activity of these various materials against specific nucleic acid synthesizing enzymes. Most recently we have been investigating the effects against DNA polymerase in an effort to localize the mechanism and site of action.

Significance to Bio-med. Research and Program of the Institute:

By understanding differences in the pharmacokinetics and pharmacodynamics of the anthracycline antibiotics, it is hoped that we will be able ultimately to understand and to define more accurately the actions of these drugs. In addition, since we have molecular windows of the various drug analogues, we may be able to envision new molecular species which can give us higher therapeutic indices.

Honors and Awards: 1971, J. D. Lane, Jr. Award, Clinical Society and Commissioned Officers Association of the United States Public Health Service.

Publications: None

Serial No. NCI-6241
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical Pharmacology of Anthracycline Antibiotics

Previous Serial Number: 6241

Principal Investigators: David Huffman and Nicholas R. Bachur

Other Investigators: Robert Benjamin
William Greene

Cooperating Units: Medicine Service, Baltimore Cancer Research Center

Man Years (July 1, 1970 to June 30, 1971)

Total:	1.4
Professional:	1.4
Other:	none

Project Description:

Objectives:

With the inclusion of the new anthracycline antibiotic, adriamycin, as an experimental cancer chemotherapeutic agent being studied by the National Cancer Institute, we foresee the future use of the anthracycline antibiotics as important chemical tools in the chemotherapy of cancer. Since we have been studying the human pharmacology of daunorubicin, we have initiated new protocols to carry out parallel studies on the similar anthracycline antibiotic, adriamycin. It is our hope that the fundamental information which we are able to uncover will be of use in the design of new anticancer drugs as well as in the scientific administration of these materials.

Major Findings:

Through the use of fluorescence assay methods developed in our laboratory, we have been successful in determining plasma levels, urinary excretion, and tissue levels of daunorubicin and its fluorescent metabolites in patients treated with this drug for disseminated solid malignancies. In this study, the patients receive daunorubicin in a single rapid intravenous dose of 80 mg/M^2 or 120 mg/M^2. The short plasma half-life as determined by fluorescence assay was 0.75 hours, and the long plasma half-life was 55 hours. The apparent volume of distribution was approximately 1000 liters with a relative volume of

distribution of 580 liters per square meter. The urinary excretion of drug and fluorescent metabolites averaged 13 to 14 per cent of the total dosage in 7 days. In addition, the daunorubicin fluorescence was detected in autopsy specimens 16 to 19 hours after drug administration with the highest levels in the kidney, spleen, liver, and lung. When drug was extracted from plasma or urine, the fluorescent species were predominantly daunorubicin, D1, and daunorubicinol, D2, although small amounts of aglycones were detected. In a parallel study when tritiated daunorubicin was used, isotopic assay produced values different from those with fluorescence assay. These were interpreted to be the result of tritium exchange or metabolism of daunorubicin. From these findings, we feel that data obtained through the use of tritiated daunorubicin is both erronious and misleading.

We have observed that daunorubicin is metabolized by a soluble enzyme in human peripheral blood elements to the metabolite D2, or daunorubicinol. Human leukocytes have a five-fold greater activity than human erythrocytes. There is no activity in carefully prepared plasma. We have also observed that leukemic leukocytes from patients with acute myeloblastic or lymphoblastic leukemia have elevated daunorubicin reductase levels.

Significance to Bio-medical Research and Program of the Institute

The anthracycline antibiotics are becoming major components in the armamentarium of the cancer chemotherapeutics. Through our studies with daunorubicin we have shown a somewhat unusual pharmacokinetic pattern of this drug which dictates to the practical pharmacology of the situation. We have therefore proposed that because of the long half-life of daunorubicin that the current treatment schedule which is by repeated doses of from 30 mg/M^2 to 60 mg/M^2 for 3 to 5 days be revised to a single dose of 120 to 180 mg/M^2 which would result in similar plasma drug levels. A second point of significance to biomedical research would be the rather unique relationship of the antibiotic daunorubicin and its metabolism to the target cell. This relationship is currently being studied and is not thoroughly understood. However, the situation exists that a drug is metabolized within the target cell to produce a metabolite which has inhibitory activity against that cell. We are currently investigating the pharmacodynamics of this situation.

Proposed Course:

This project is to be continued and expanded.

Honors and Awards: None

Publications:

Alberts, D., Bachur, N. R., and Holtzman, J.: The Pharmacokinetics of Daunomycin in Man. Clin. Pharmacol. and Therapu., 12: 96-104, 1971.

Serial No. NCI-6271
1. Baltimore Cancer Research Center
2. Biochemistry Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Nuclear Phosphoproteins

Previous Serial Number: None

Principal Investigator: Khalil Ahmed

Other Investigators: Hajime Ishida

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971)

Total:	0.7
Professional:	0.7
Other:	none

Project Description:

Objectives:

The thorny problem of gene activation is of obvious relevance to the question of tumor host relationship. An experimental approach to this problem is the use of model systems in which cell proliferation can be induced by physiological or pathological stimuli. This laboratory is using testosterone stimulation of prostate, and isoproterenol stimulation of salivary glands as the two model systems to investigate the changes induced in nuclear phosphoprotein metabolism. The study of phosphoprotein metabolism, with a view to establish the physiological role of these molecules in the cellular machinery, has been of particular interest to us for several years. During the past few years, great interest has been aroused in the metabolism of nuclear phosphoproteins with the possibility that they may be involved in the processes related to gene activation. We are, therefore, pursuing the study of the two model systems, described above, from such a point of view.

Methods Employed:

Suitable methods for the isolation of nuclei were developed for these studies. ^{32}P-labeled ATP is synthesized and incorporation of ^{32}P into phosphoproteins is investigated in normal and treated rats. The methods for the isolation of phosphoproteins have been described by us.

Major Findings:

We have demonstrated that nuclear phosphoproteins from prostate and salivary glands incorporate ^{32}P at very rapid rates. The phosphorylation is optimal in the presence of Mg^{2+} and Na^{+} but is inhibited by Ca^{2+}. A large portion of the radioactivity is present in the so-called non-histone fraction. Orchiectomy of rats leads to a large decline in the rates of incorporation of ^{32}P, which is prevented by injections of testosterone. A relationship to the control of gene action is shown from our observation that testosterone stimulates the phosphorylation as early as 30 mins after the injection at a time when intense gene activity is taking place in the prostate. The possibility that a stimulation of the enzymes involved is a strong one and is also supported by some preliminary data on isoproterenol-stimulated salivary glands.

Significance to Bio-med. Research and Programs of the Institute:

The above observations are of potential importance in the study of the mechanism of hormone action and in furthering our knowledge of events related to gene action.

Proposed Course:

We hope to continue the investigations related to nuclear phosphoprotein metabolism using isoproterenol stimulation of salivary glands and hyperplastic prostate following prolonged testosterone treatment as two models of cellular proliferation. The underlying biochemical mechanisms will be explored such as the role of cyclic AMP and cation shifts which might influence the phosphorylation reactions.

Honors and Awards: None

Publications:

K. Ahmed: Effect of Testosterone on Nuclear Phosphoproteins of Rat Ventral Prostate. Pharmacologist, 12: 229, 1970.

K. Ahmed: Studies on Nuclear Phosphoproteins of Rat Ventral Prostate: Incorporation of ^{32}P from γ-^{32}P-ATP. Biochim. Biophys. Acta, in press.

K. Ahmed and H. Ishida: Effect of Testosterone on Nuclear Phosphoproteins of Rat Ventral Prostate. Molec. Pharmacol., May 1971.

Serial No.: NCI-6210
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Development of Specific RNAses as an Aid in the Sequencing of t-RNAs from Ehrlich Ascites Cells

Principal Investigator: C. C. Levy

Other Investigators: W. Mitch
S. Friedling

Cooperating Units: None

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1/2
Professional:	1/2
Other:	0

Project Description:

Objectives

The isolation from microbial sources of a nuclease specific for Poly IC as an aid in the assay and identification of this polymer.

The isolation from microbial sources of nucleases which will hydrolyze nucleic acids at specific linkages as an aid in the sequential analysis of t-RNA from various tumor or viral sources.

Major Findings

The technique of enrichment culture was employed to isolate four microorganisms from soil which could degrade RNA. Each organism elaborates a different type of RNAse. One is an endonuclease which is quite specific for cytidylic acid internucleotide linkages and deposits 2'3' cyclic CMP as the end product of hydrolysis of the nucleotide chain. The other, an exonuclease, is specific for adenylic acid internucleotide linkages. The terminal product of its catalysis is 5' AMP. Both enzymes require metals (cobalt and magnesium respectively), and are relatively stable with rather high molecular weights.

An additional finding of some interest is that the endonuclease which has specificity for cytidylic internucleotide linkages appears to have cytotoxic properties towards cultures of L_{1210} leukemia cells. Currently, large amounts of enzyme are being isolated so that this property can be tested towards a variety of tumor cells.

The remaining organisms (2) appear to produce ribonucleases which are specific for uridylic and guanylic acid internucleotide linkages. The properties of these enzymes are currently under investigation.

Significance to Cancer Research

Nucleic acid metabolism or, more correctly, the alteration of nucleic acid metabolism is believed to play an important role in cancer induction. It is hoped that an understanding of the sequential relationship of one nucleic acid to the other in the construction of a large chain polymer from tumor cells may provide a clue as to the nature of cancer induction.

Projected Course

To continue in the isolation of more specific nucleases and to begin the isolation of homogeneous t-RNA from tumor and/or viral sources.

Honors and Awards: None

Levy, C. C. and Goldman, P.: Residue Specificity of a Ribonuclease which Hydrolyzes Polycytidylic Acid. J. Biol. Chem. 245, 3257-3262, 1970.

Russell, D. H. and Levy, C. C.: Polyamine Accumulation and Biosynthesis in a Mouse L_{1210} Leukemia. Cancer Res. 31, 248-251, 1971.

Serial No.: NCI-6242
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Polyamine Synthesis in Rapidly Growing Tissues (Normal and Neoplastic)

Principal Investigator: D. H. Russell

Other Investigators: C. C. Levy
M. J. Feldman

Cooperating Units: None

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1
Professional:	1
Other:	0

Project Description:

Objectives
To define the function of putrescine, spermidine, and spermine, and to understand possible control mechanisms of their synthesis.

Methods Employed
1. Determination of putrescine, spermidine, and spermine in tissues by homogenization in acid, alkaline butanol extraction and high voltage electrophoresis for separation.
2. Ornithine decarboxylase activity (putrescine-forming enzyme) determined by liberation of $^{14}CO_2$ from ornithine-1-^{14}C.
3. S-adenosyl-L-methionine decarboxylase activity (spermidine-forming enzyme) determined by $^{14}CO_2$ release from S-adenosyl-L-methionine and from ^{14}C-spermidine formation from ^{14}C-putrescine.
4. Determination of RNA polymerase activity in nucleolar preparations and the effect of various polyamines on this activity.
5. Routine determination of the relationships between RNA content and polyamine concentrations.

Major Findings
A study conducted during the last year indicates that patients with diagnosed cancers have elevated urinary polyamine levels. This is based upon the evaluation of 75 cancer patients and 40 controls. In leukemias, the excretion level declines when the patients are in remission. After surgery for the removal of a portion of a solid tumor mass, polyamine levels in the urine decreased to near normal in all cases. It is possible, therefore, that poly-

amine excretion levels could serve as (1) a tool to monitor malignancy and (2) a test that could lead to an early detection of malignancy. Further studies as follows are planned, but some of these projects will need to be contracted to private agencies.

a) Continued screening of diagnosed cancer patients to further establish the validity of these findings.

b) Regular screening of AML's before, during, and after chemotherapy, to determine if polyamine levels at any given point in therapy can serve as a reliable indicator for future chemotherapy, i.e. best possible dosage schedule, when to stop, when to continue chemotherapy.

c) Regular screening at a clinic such as the Bon Secours breast tumor unit. Polyamine levels could be ascertained prior to biopsy and correlated with biopsy results, i.e. malignant or benign.

d) It would be useful to obtain data on a group of individuals who have a high incidence of cancer. This could establish whether polyamine levels increase in the early initiative stages of cancer, before cancers can be detected histologically. One such group might be the uranium miners in Colorado. Another possibility might be to screen a retirement community.

e) Screening of urines of patients with known cancer histories. This could build up a body of data as to whether elevated polyamines indicate renewed malignancy. If so, early treatment could save more lives.

f) A possible metabolite of spermidine has been detected in the urine of both normals and experimentals. This substance is much higher in the urine of cancer patients. This compound should be identified and the enzyme(s) system which is responsible for its formation isolated. This enzyme could be potentially useful to lower spermidine levels in tumors.

g) Two inhibitors of ornithine decarboxylase have been found, α-methyl ornithine and N-5-ornithine. Extensive supervised screening of these compounds should be initiated.

Considerable data have been accumulated concerning the effects of polyamines on nucleolar RNA polymerase activity. Nucleolar RNA polymerase (RNA polymerase I) was partially purified from rat liver nucleolar preparations and stabilized by the addition of albumin. The techniques as described by Roeder and Rutter (PNAS 65: 675, 1970) were used. After removing the enzyme from DEAE-sephadex, the preparation was dialyzed to remove any cations. It was observed that there was a 2-3 fold increment in RNA polymerase I activity when the enzyme was assayed in the presence of spermidine as opposed to that found in the presence of magnesium. In addition to the stimulation of RNA polymerase I activity, the polyamines caused structural alterations in the nucleolus. Electron micrographs showed definitive changes in the nucleoli when the nuclei were isolated in the presence of 2.5-10 mM putrescine, spermidine, or spermine, in contrast to that seen with similar concentrations of magnesium. Nuclei isolated by the addition of either spermidine or spermine as the stabilizing cation had nucleoli that were significantly larger than those isolated with magnesium. In general, when the nuclei were isolated in the presence of magnesium, the nucleoli were compact and electron dense, whereas in the presence of spermidine or spermine, the nucleolonemas were less aggregated, and the nucleoli contained lacunae. Both spermidine and spermine (10 mM) caused the formation of ring-shaped nucleoli.

The enzyme, S-adenosyl-L-methionine decarboxylase, has been purified to near

homogeneity as evidenced by acrylamide gel electrophoresis, sucrose density centrifugation and sephadex gel filtration. Attempts at ammonium sulfate fractionation of the crude extract resulted in a 90% loss of the enzyme activity. However, by the omission of the ammonium sulfate step and the use of sephadex gel filtration and DEAE-cellulose, we recovered 90% of the original activity. This activity was stabilized by the addition of 1 mM dithiothreitol, 1 mM EDTA, and 2μM pyridoxal phosphate. In the presence of these substances there was no significant loss of enzyme activity over a 3-month period at -20°. Estimation of the molecular weight, both by sephadex gel filtration and sucrose density centrifugation, gave an average of 45,000. The purified enzyme was sensitive to low concentrations of p-chloromercuribenzoic acid and n-ethyl maleimide, known inhibitors of sulfhydral groups. Future plans will include purification of this enzyme from malignant tissues and determination of control mechanisms, i.e. are they the same or different from normal tissue controls?

Significance

Much progress has been made in terms of the elucidation of the functional role and/or roles of polyamines. This progress brings us closer to an understanding of the control mechanisms involved in the growth process.

Publications

Snyder, S. H., Kreuz, D. S., Medina, V. J., and Russell, D. H.: Polyamine Synthesis and Turnover in Rapidly Growing Tissues. Ann. N. Y. Acad. Sci. 171: 749-771, 1970.

Russell, D. H.: Putrescine and Spermidine Biosynthesis in Growth and Development. Ann. N. Y. Acad. Sci. 171: 772-782, 1970.

Russell, D. H., Medina, V. J. and Snyder, S. H.: The Dynamics of Synthesis and Degradation of Polyamines in Normal and Regenerating Rat Liver and Brain. J. Biol. Chem. 245: 6732-6738, 1970.

Russell, D. H. and Levy, C. C.: Polyamine Accumulation and Biosynthesis in a Mouse L_{1210} Leukemia. Cancer Res. 31: 248-251, 1971.

Russell, D. H.: Putrescine and Spermidine Biosynthesis in the Development of Normal and Anucleolate Mutants of Xenopus laevis. Proc. Natl. Acad. Sci.U.S.A. 68: 523-527, 1971.

Russell, D. H. and Taylor, R. L.: Polyamine Synthesis and Accumulation in the Castrated Rat Uterus after Estradiol 17-β Stimulation. Endocrinology, in press.

Russell, D. H. and Lombardini, J.: Polyamines: (1) Enhanced S-adenosyl-L-methionine decarboxylase in rapid growth systems, and (2) the Relationships Between Polyamine Concentrations and RNA Accumulation. Biochim. Biophys. Acta, in press.

Russell, D. H., Shiverick, K. T., Hamrell, B. B. and Alpert, N. R.: Polyamine Synthesis During the Initial Phases of Stress-induced Cardiac Hypertrophy. Amer. J. Physiol., in press.

Serial No.: NCI-6243
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Heterogeneity and Molecular Transformations of Glucose-6-Phosphate Dehydrogenase

Principal Investigator: M. Schmukler

Other Investigators: None

Cooperating Units: None

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1/8
Professional:	1/8
Other:	0

Project Description:

Objectives
To determine factors involved in the heterogeneity of glucose-6-phosphate dehydrogenase.

Major Findings
Three distinct enzymatically active glucose-6-phosphate dehydrogenases are detectable on polyacrylamide gel electrophoresis. On study, it was demonstrated that these enzymes were members of a polymeric series with molecular weights in the ratio of 1:2:3. Sulfhydryl reagents cause a shift to the monomer while oxidants encourage the formation of polymers.

Significance to Cancer Research
Factors involved in the alteration of structure and/or function of protein molecules could play a role in the production of malignant transformations. They could also suggest mechanisms whereby cellular function could be altered, leading to de-differentiation of the cell.

Proposed Course - Study completed.

Publication

Schmukler, M.: The Heterogeneity and Molecular Transformations of Glucose-6-Phosphate Dehydrogenase of the Rat. Biochim. Biophys. Acta 214, 309-317, 1970.

Serial No.: NCI-6244
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Fatty Acid Synthesis by Human Platelets

Principal Investigators: M. Schmukler
P. Zieve

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives
To study the control of fatty acid synthesis in human platelets and to note similarities to and differences from other tissues.

Major Findings
When washed human platelets are incubated in vitro, glucose stimulates fatty acid synthesis. By use of inhibitors of glycolysis, the monophosphate shunt, and the Krebs cycle, it was concluded that the effect of glucose required the operation of both the shunt and the Embden-Myerhoff pathway.

Significance to Cancer Research
Control of hemorrhage is a major problem in leukemia and in patients receiving cancer chemotherapy. Platelet transfusions are often needed to stop bleeding. The viability of platelets is dependent upon their continued normal metabolism, so that factors involved in the control of platelet metabolism must be delineated in order to preserve platelet viability for transfusions.

Proposed Course
Studies on control factors in lipid metabolism will be pursued, particularly with regard to factors involved in stabilization of platelets during storage.

Publications
Zieve, P. and Schmukler, M.: The Stimulation by Glucose of Fatty Acid Synthesis in Human Platelets. Amer. J. Physiol. 219: 1009, 1970.

Serial No.: NCI-6245
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Human Platelet Metabolism: The Effect of Cyclic AMP

Principal Investigators: P. Zieve
M. Schmukler

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives
To study the metabolism of platelets in order to define, if possible, the biochemical factors involved in platelet aggregation.

Major Findings
Agents which initiate platelet aggregation all cause a marked stimulation in platelet lactate production. Cyclic AMP, an inhibitor of aggregation, stimulates conversion of glycogen to glucose in the platelet, while at the same time inhibiting lactate production. The mechanism whereby this occurs was studied in detail and a single site of inhibition was located.

Significance to Cancer Research
Platelet aggregation is one of the factors involved in the clotting mechanism. Hemorrhage is one of the major problems in cancer chemotherapy and is often controlled by platelet transfusion. Studies of the biochemistry of platelet aggregation should contribute significantly to the intelligent storage and use of platelets in control of bleeding.

Proposed Course
Studies on the metabolic changes occurring under conditions which favor or inhibit platelet aggregation will continue.

Publications - None

Serial No.: NCI-6246
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Humoral Control of Fatty Acid Synthesis by Human Platelets

Principal Investigators: M. Schmukler
P. Zieve

Other Investigators: None

Cooperating Unit: Division of Hematology, Baltimore City Hospitals

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives
To study mechanisms involved in the control of fatty acid synthesis in human platelets and to study evidence suggesting the existence of a humoral factor which stimulates lipogenesis.

Major Findings
Platelets isolated from individuals who have been fed glucose incorporate acetate into fatty acids more rapidly than do control platelets. While a similar effect was noted after fructose feeding, galactose feeding had no effect. Preincubation of whole blood with glucose prior to platelet isolation did not stimulate fatty acid synthesis. Glycogen and lactate levels and rates of lactogenesis, glycogenolysis, and glucose oxidation were not affected by glucose feeding.

Significance to Cancer Research
Major causes of death in leukemia are infection and hemorrhage. These are also complications of chemotherapy. The platelet plays a major role in the clotting mechanism and platelet transfusions are used to control hemorrhage in cancer patients. Knowledge of the metabolism of platelets and of factors involved in platelet stability are therefore necessary to allow effective use of these cells in control of bleeding.

Proposed Course
The effects of other commonly ingested substances will be studied in this system to delineate, if possible, the essential chemical structure necessary to produce the lipogenic effect.

Serial No.: NCI-6247
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Purification of Bacterial Nucleases with Residue Specificity

Principal Investigator: M. Schmukler

Other Investigator: C. C. Levy

Cooperating Units: None

Man Years: (July 1, 1970 through June 30, 1971)

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives
Purification of a ribonuclease from Klebsiella species.

Major Findings
A ribonuclease from Klebsiella grown on deoxy AMP or DNA as a nitrogen source has been purified 550-fold. It shows a specificity for poly A and poly U, artificial polymers but is able to digest ribonucleic acid completely, releasing 2'-3' cyclic mononucleotides. The enzyme has no cation requirement and has a pH optimum at 7.0 in phosphate and 7.5 in Tris.

Significance to Cancer Research
The study of enzymes concerned with nucleic acid metabolism is essential to an understanding of the mechanisms of carcinogenesis and cancer control since there is obviously an alteration in nucleic acid metabolism in cancer and since most chemotherapeutic agents are directed in some way against nucleic acid metabolism. Nucleases which have some residue specificity should be valuable tools in the delineation of nucleic acid structure. It is also possible that nucleases with some type of specificity may be useful as chemotherapeutic agents.

Proposed Course
After stabilizing enzyme at its present purity, specificity studies, kinetics and anticancer properties will be studied.

Publications - None

Serial No.: 6258
1. BCRC
2. Enzymology and Drug Metabolism
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Glycosidase Levels in Serum, White Cells and Marrow of Leukemic Patients

Principal Investigators: M. Schmukler
J. Holtzman

Other Investigators: None

Cooperating Unit: None

Man Years: (July 1, 1970 through June 30, 1971)

Total:	3/8
Professional:	3/8
Other:	0

Project Description:

Objectives
1. To see whether there is a correlation between existence of malignancy and glycosidase levels.
2. To see whether glycosidase levels correlate with presence or absence of drug resistance.
3. To see whether glycosidase levels reflect remission or relapse.

Major Findings
Techniques to the study are being set up. Umbelliferone substrates are being synthesized.

Significance to Cancer Research
Data from this study may aid in the detection of malignancy and may detect the onset of drug resistance, thereby helping the chemotherapist to develop a more rational regimen.

Proposed Course
Assays will be done on material from every new leukemic patient on admission, and at periodic intervals after initiation of therapy.

Publications - None

Serial No. NCI-6019

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Search for the Presence of Tumor Specific Antigens on Human Neoplastic Cells.

Previous Serial Number: 6019

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Steven P. Kanner, M.D.
Richard J. Mangi, M.D.
Gerald N. Bock, M.D.
Leonard Chess, M.D.

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971)

Total:	1 year
Professional:	2/3
Other:	1/3

Project Description:

Objectives:

To develop systems to define antigenic differences between normal cells and autochtonous tumor.

Methods Employed:

Assays were performed in which the response of murine and human lymphocytes to x-irradiated syngeneic tumors were conducted in vitro by a modification of the mixed lymphocyte reaction. Tritiated thymidine incorporation, cell number, lymphocyte transformation and autoradiographic analysis were used as measures of response. In the human system, an additional format of design (third man test) was employed in which frozen-stored pre and post therapy CLL cells were evaluated as stimulators in a one-way mixed lymphocyte reaction against a battery of lymphocytes from normal allogeneic individuals. If the pre and post therapy lymphocytes possessed quantitatively and qualitatively similar antigens, one would expect identical thymidine incorporation to each population by normal allogeneic responding lymphocytes (J. Exp. Med. 132:401, 1970).

Major Findings:

1. C57BL/6 lymphocytes were shown to respond to B16 melanoma in the mixed lymphocyte tumor reaction. The kinetics of response were identical to the normal murine mixed lymphocyte reaction. Similarly, DBA/2 lymphocytes were shown to respond to P388 lymphoma.
2. Human lymphocytes from 2 patients with malignant melanoma were found to respond to autochtonous tumor.
3. Pre and post therapy frozen stored CLL cells were found to differ qualitatively or quantitatively in their ability to act as antigens in the one-way MLR.

Proposed Course:

To maximize the ability to measure host response to tumors in these systems.

Honors and Awards: None

Publications:

Mangi, R.J., Weiner, R.S. and Mardiney, M.R., Jr.: Transformation Response of Frozen-Stored Chronic Lymphocytic Leukemia Cells: A Preliminary Report. European J. of Clin. and Biol. Res., to be published, June, 1971.

Kanner, S.P., Mardiney, M.R., Jr., and Mangi, R.J.: Experience with a Mixed Lymphocyte Tumor Reaction as a Method of Detecting Antigenic Differences between Normal and Neoplastic Cells. J. Immunol. 105:1052, 1970.

Serial No. NCI-6020

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanisms of Human Lymphocyte Reactivity to Non-Self.

Previous Serial Number: 6020

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Richard J. Mangi, M.D.

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971)

Total:	1 year
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

To determine the sensitivity of the mixed leukocyte reaction and to develop a murine model system in which cell genetics could be uniquely controlled.

Methods Employed:

Short-term tissue cultures in which the proliferative response of cells in question were monitored by measuring the incorporation of thymidine. Evaluation of the sensitivity of the MLR was performed by using congenic resistant strains and a background strain differing at only one locus (C57 BL/10ScSn). Correlation of the MLR responses to known skin graft rejection times in these animals were made.

Major Findings:

Contrary to past belief (Science 156:1506, 1967), it was defined that the mixed lymphocyte reaction could be stimulated by differences at single minor histocompatibility loci. Regression analysis of the correlation of the MLR data and corresponding median skin graft survival times indicated an index of determination of 0.87. The equation derived relating MLR and skin graft survival was $Y = a + b/X$.

Proposed Course:

To attempt to maximixe the MLR in man in order to enable to detect cells which differ at minor histocompatibility loci.

Honors and Awards: None

Presentation:

Third International Congress of the Transplantation Society.

Publications:

Mangi, R.J. and Mardiney, M.R., Jr.: The Sensitivity of the Mixed Lymphocyte Reaction and its Correlation with Skin Graft Survival. Transplantation Proc., in press, February, 1971.

Mangi, R.J. and Mardiney, M.R., Jr.: The Mixed Lymphocyte Reaction: Detection of Single Histocompatibility Loci and the Correlation to Skin Graft Survival in Mice. Transplantation, in press, April, 1971.

Mangi, R.J. and Mardiney, M.R., Jr.: The Sensitivity of the Mixed Lymphocyte Reaction and its Correlation with Skin Graft Survival. Abstracts, Third International Congress of tee Transplantation Society, Printed in the Netherlands by N.V. Zuid-Hollandsche Boek- en Handelsdrukkerij, The Hague, Abstract No. 93, 1970.

Serial No. NCI-6248

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: To Determine the Effect of Freezing on the Transformation of Lymphocytes in the Mixed Lymphocyte Reaction and in Response to Specific Antigens and PHA.

Previous Serial Number: 6248

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Richard J. Mangi, M.D.
Gerald N. Bock, M.D.
Leonard Chess, M.D.

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971)

Total:	1 year
Professional:	1/2
Other:	1/2

Project Description:

Objectives:

Previous work had defined that cells frozen at different times and stored for various times transform in a reproducible manner when placed in culture with PHA, as one population of the two-way or the one-way mixed lymphocyte reaction and that frozen-stored lymphocytes responded minimally when cultured with specific antigen, or as both partners in the mixed lymphocyte reaction. Continued experimentation with this system was designed to define those parameters whereby frozen lymphocytes would be made to respond in situations of minimal antigen difference.

Methods Employed:

1. Slow freezing of lymphocytes in media containing 10% DMSO.
2. Short-term tissue cultures in which the proliferative response of the cells in question were evaluated by measuring the incorporation of thymidine.

Major Findings:

Reconstitution of frozen lymphocyte reactivity could be made simply with fresh autologous or homologous plasma. Fresh granulocytes or monocytes could not reconstitute this activity.

Proposed Course:

To study the response of lymphocytes to tumor cells before, during, and after the institution of therapy.

Honors and Awards: None

Publications:

Mangi, R.J. and Mardiney, M.R., Jr.: The In Vitro Transformation of Frozen-Stored Lymphocytes in the Mixed Lymphocyte Reaction and in Culture with Phytohemagglutinin and Specific Antigens. J. Exp. Med. 132:401, 1970.

Mangi, R.J. and Mardiney, M.R., Jr.: Purification of Human Lymphocytes by Simple Centrifugation. European J. of Clin. and Biol. Res. 15:911, 1970.

Serial No. NCI-6249

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Search for Antigen-Antibody Complexes in Renal Glomeruli of Tumor Patients.

Previous Serial Number: 6249

Principal Investigator: John C. Sutherland, M.D.

Other Investigators: Michael R. Mardiney, Jr., M.D.

Cooperating Units: Department of Pathology, U.S.P.H.S. Hospital, Baltimore, Maryland 21211

Man Years: (July 1, 1970 to June 30, 1971)

Total:	1 year
Professional:	1/2
Oteer:	1/2

Project Description:

Objectives:

To determine whether long-term viral disease is the etiology of some forms of neoplasia in man.

Methods Employed:

Post-mortem kidneys obtained from tumor and non-tumor patients and renal biopsies from patients with Hodgkin's disease and lymphosarcoma undergoing laparotomy for staging were screened for immunologic injury to renal glomeruli by fluorescent, light and electron microscopy. Those with evidence of entrapped complexes were further studied using a battery of anti-viral sera to 11 different viruses of known or suspected oncogenicity. 201 post-mortem kidneys and 15 renal biopsies were studied.

Major Findings:

Gamma globulin was found entrapped in the glomeruli of 24 post-mortem kidneys, 19 from patients with tumor and 5 from patients without tumor. Eleven of these have also been tested for complement and complement was found in 4. Of the 15 renal biopsies, gamma globulin associated with complement was found

in 4 and complement alone in another 2. In 2 kidneys electron dense deposits have been found on glomerular basement membranes. Studies to attempt to identify the antigen in these complexes have been completed or partially completed in 11 kidneys, 8 of those taken post-mortem and 3 of the renal biopsies. Nine of these have been negative when tested with a battery of 11 anti-viral sera. Positive fluorescence was demonstrated with antiserum to Mycoplasma pneumoniae in a post-mortem kidney from a patient with acute myelomonocytic leukemia and with antiserum to the cat leukemia virus in another post-mortem kidney from a patient with acute myelocytic leukemia. Homogenizing the whole kidney from the latter patient we have recovered antibody by acid elution and antigen by sonication of the renal precipitate. These give a strong band of precipitation when tested against each other in gel diffusion and this band is continuous with bands formed between cat leukemia viral antigen and antibody from the kidney, and between cat leukemia viral antigen and cat leukemia viral antibody of rabbit origin.

Proposed Course:

1. To continue to screen for antigen in these complexes
2. To continue to elute antibody from these kidneys and test for specificity.
3. To continue screening patients for this type of injury.

Honors and Awards: None

Publications: None

Serial No. NCI-6273

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Bacterial Contamination in Platelet Products Stored at 25° C.

Previous Serial Number: None

Principal Investigator: D. H. Buchholz, M.D.

Other Investigators: Viola Mae Young, Ph.D.
Michael R. Mardiney, Jr., M.D.

Cooperating Units: Central Blood Supply; Baltimore Red Cross

Man Years:

Total:	7/10
Professional:	1/5
Other:	1/2

Project Description:

Objectives:

Recent reports have suggested improved post-transfusion platelet survival if platelet products are prepared and stored at 25° C rather than 4° C as has been done previously. An objection to 25° C storage is the possibility of proliferation of bacteria introduced inadvertently into blood collection systems during phlebotomy or component preparation; such bacteria might multiply much more rapidly at the higher storage temperature. Following two instances of gram-negative septicemia in cancer patients transfused with such stored platelets, a study was begun to determine the frequency of bacterial contamination in platelets stored at 25° C.

Methods Employed:

Platelet concentrates were obtained from commercial and volunteer sources and were pooled in groups of 8 to 10 units after variable periods of storage at ambient temperature for up to 96 hours. Each pool was composed of platelets obtained from a common source and stored a similar length of time prior to culture. A sample of each pool was inoculated into tryptic soy broth, brain heart infusion and thioglycollate broth; the three media were incubated at 37° C. Later in the study duplicate cultures were additionally performed at

25° C. Precautions were taken to prevent contamination during pooling or culturing. Cultures were examined frequently for evidence of bacterial growth; all apparently negative cultures were routinely entered and subcultured on two occasions. Bacteria were identified according to standard methods.

Major Findings:

Culture of 2188 units of platelets in 258 pools revealed the presence of bacteria in 53 pools, an incidence of 20 per cent. While diphtheroids and Staphylococcus epidermidis were isolated most frequently, other bacteria including species of Micrococcus, Streptococcus, Sarcina, Bacillus, Herellea, Pseudomonas and Flavobacterium were also found. Enterobacter cloacae was isolated seven times and caused both septicemias. If it is assumed that only one unit of any contaminated pool contributed all bacteria, 2.4 per cent of commercial and 2.5 per cent of volunteer platelets contained microorganisms. There was no significant difference in contamination of units prepared by plasmaphoresis, as opposed to single unit donations, although more contamination was noted in units stored as platelet rich plasma than platelet concentrate. The recovery of bacteria was significantly increased with increased time of storage at 25° C, suggesting proliferation of existing microorganisms during storage. No evidence of contaminated blood collection bags or supplies used in donor arm cleansing could be found. Since many of the bacteria found were typical skin flora, it was felt that bacteria in hair follicles or sebaceous glands that might not be killed by even vigorous donor arm cleansing might account for the source of contamination if donor skin were "cored" by the needle during venipuncture. Studies are currently underway to evaluate this possibility.

Proposed Course:

Room temperature storage of platelets significantly improves post-transfusion survival compared with storage at 4° C. At the latter temperature, platelets clump irreversibly and are rapidly cleared from the circulation after infusion. The finding of bacterial contaminants in 2.5 per cent of platelets stored at 25° C, however, raises serious questions as to whether the benefits of increased platelet survival outweigh the risk of bacterial proliferation. By adding extra ACD anticoagulant to platelet concentrate, platelet clumping at 4° C can be diminished. We plan to investigate the incidence of bacterial contamination in acidified platelets stored at 4° C in an effort to decrease the risk of bacterial contamination in platelet transfusions without seriously compromising platelet survival after transfusion.

Honors and Awards: None

Publications:

Buchholz, D.H. and Young, Viola M.: Bacterial Contamination in Platelets Stored at Room Temperature. Proc. Amer. Assoc. for Cancer Res., 12:76, 1971.

Serial No. NCI-6274

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Production of Tumor Specific Antibody in a Xenogeneic Species Utilizing Blocking Antibody.

Previous Serial Number: None

Principal Investigator: Michael R. Mardiney, Jr., M.D.

Other Investigators: Roy S. Weiner, M.D.
Jeffrey D. Hubbard, M.D.
Donald H. Buchholz, M.D.
Peter Mancusi-Ungaro, M.D.

Cooperating Units: None

Man Years (July 1, 1970 to June 30, 1971

Total:	1 1/2
Professional:	1
Other:	1/2

Project Description:

Objectives:

One of the main obstacles in tumor immunology is the inability to differentiate the presence or absence of antigens upon neoplastic cells which are qualitatively or quantitatively specific for that cell. This has in part been the problem even in a species such as the mouse in which the ability for repeated immunologic analyses are great. Previous attempts to produce antibody against tumor-specific antigens especially in non-viral systems have been difficult. Although antibody specific for murine tumor antigen may be obtained in a homologous host, it is not the rule. Attempts to produce such antibody may be attended by repeated failure before success is achieved. In addition, the situation has been almost hopeless if a host outside the species is used as a source of antibody. We recently have utilized a method for the development of antibody to tumor cells which has bypassed some of the problems described. The technique is based upon the principle that passively administered antibody can specifically inhibit production of similar antibody within hosts of the same species or outside that species. The most practical clinical application of the blocking phenomenon is the prevention of erythroblastosis fetalis by the suppression of the development of antibody in the Rh negative mother through injection of anti

Rh containing immune globulin within 3 days of delivery of an Rh positive baby. In an effort to develop a model of antibody production against tumor antigens which could be applicable to the clinical situation, we decided to define the parameters whereby a heterologous species - namely the rabbit - could be modulated to produce antibody specific for a murine leukemia. The leukemia utilized as the target for the experiment was the EL4, a methyl cholanthrene induced tumor of the C57 black mouse. Previous attempts at inducing specific antibody production in the rabbit using either purified or unpurified populations of this cell had met with failure. An attempt was made to selectively suppress antibody production in the rabbit to antigens common to both normal and EL4 cells. If this could be accomplished then perhaps antigenic competition would also be inhibited - enhancing the chance that the rabbit would respond to tumor specific antigens.

Methods Employed;

Normal New Zealand rabbits were immunized to C57BL/6 cellular antigens. This antibody was then incubated in excess with EL4 leukemia cells. Both antibody and cells were then transferred into new normal New Zealand rabbits. The antibody derived from these animals were analyzed for specificity to EL4.

Major Findings:

The antibody produced had a marked differential of 50% cytotoxicity to normal C57 lymphocytes (1:40 to 1:80) as compared to EL4 cells (1:2000). Eradication of the specificity of its antibody response to EL4 by absorption with C57BL/6 lymphocytes revealed that this antibody could not be absorbed with normal C57BL/6 lymphocytes nor could its activity be absorbed by in vivo absorption in the C57BL/6 mouse.

Significance:

This approach has allowed for the modulation of the antibody response of a heterologous species to cellular antigens and may represent the much needed method for the analysis of these antigens.

Proposed Course:

To determine applicability of this approach on a broader scale. To evaluate therapeutic uses of such antibodies.

Honors and Awards: None

Presentation:

Mardiney, M.R., Jr. , Department of Medicine, Johns Hopkins Medical School, April 30, 1971.

Presentation (con't)

Weiner, R.S., American Association for Cancer Research, April 10, 1970.

Publications:

Weiner, R.S., Hubbard, J.D. and Mardiney, M.R., Jr.: Production of Xenogeneic Anti-Tumor Antibody by Employing Blocking Antibody. Proc. Amer. Assoc. for Cancer Res., 12:76, 1971. Abstract No. 302.

Serial No. NCI-6275

1. Baltimore Cancer Research Center
2. Immunology and Cell Biology
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cell Transfusion Therapy

Previous Serial Number: None

Principal Investigator: D. H. Buchholz, M.D.

Other Investigators: Michael R. Mardiney, Jr., M.D.

Cooperating Units: Baltimore Red Cross; Central Blood Service

Man Years:

Total:	1 3/4
Professional	1/2
Other:	1 1/4

Project Description:

Objectives:

With the advent of newer aggressive chemotherapeutic approaches to the treatment of malignancy, problems arising from secondary suppression of the bone marrow have become more prominent. Consequent to chemotherapy, many patients become severely granulocytopenic and thrombocytopenic, leading to greatly increased risk of infection or hemorrhage. Replacement therapy consisting of granulocyte and platelet transfusion should substantially decrease these patient risks.

Methods Employed:

I. Platelet Transfusion

Platelets are supplied from the Baltimore Red Cross and from the Central Blood Service. Recent reports have suggested that preparation and storage of platelets at room temperature (25° C), rather than conventional blood bank refrigerator temperatures (4° C), offers significant improvement in post-transfusion platelet survival at times when fresh platelets are not readily available. Evaluation of therapeutic effectiveness of such platelets, compared with those frozen in liquid nitrogen with DMSO or those stored at 4° C, is currently underway.

II. White Blood Cell Transfusion

Granulocytes may be obtained from donors through the use of the NCI-IBM Cell Separator. This machine semi-selectively separates granulocytes from donor blood by means of differential centrifugation by repeatedly circulating donor blood through the machine. Large numbers of donor granulocytes may be removed and transfused to recipients to help prevent or to treat infection. In order to prevent transfusion reactions and to insure improved post-transfusion granulocyte survival in recipients, family members other than non-relatives are utilized as donors. All family members are histocompatibility (HL-A) typed to insure the selection of the donor (s) most compatible with (or identical to) the recipient.

Major Findings:

Platelet Transfusion

Platelet infusions prevent or control bleeding due to thrombocytopenia and play an important role in the supportive treatment of cancer patients. Splenomegaly and elevated temperatures are detrimental to good post-transfusion platelet survival. Room temperature storage of platelets for use when fresh platelets are not readily available has been shown to provide adequate patient platelet levels after storage for up to 48 hours. Although limited comparisons have been made, room temperature storage seems significantly better than storage at 4° C and much better than freezing platelets in DMSO and liquid nitrogen. Since only 1/4 to 1/5 the number of room temperature stored platelets are needed for transfusion, (compared with the number required when frozen platelets are used), there is a decreased risk of recipient hepatitis and sensitization to platelet antigens.

White Blood Cell Transfusion

Granulocyte transfusions have been given both for prophylaxis against infection and for treatment of documented septicemia. All donors have been HL-A typed and selected to be those most compatible with the recipient. From 3.1×10^9 to 11×10^9 granulocytes have been obtained during single donations. Transfusion of these cells has not significantly raised recipient WBC at either 1 or 12 hours after infusion but has provided circulating granulocytes, as can be demonstrated on peripheral blood smear, with granulocytes increased from 0 to as high as 35% of circulating WBC. Insufficient data is available at the present time to adequately evaluate the role granulocyte transfusion plays in the supportive treatment of cancer patients.

Proposed Course:

Platelet Transfusion Program

1. Evaluation of methods of platelet storage will continue. Storage at 4° C using excess ACD anticoagulant to prevent platelet clumping and to prolong platelet life span will be evaluated in a controlled study.

2. A plateletphoresis program utilizing HL-A typed family members as bi-weekly platelet donors will be instituted in an attempt to provide compatible platelets to patients with platelet antibodies which destroy random donor platelets.

WBC Transfusion

1. Increased use of granulocyte transfusion will be made; the efficiency of such collected granulocytes will be evaluated by clinical observation, as well as in vitro techniques to evaluate phagocytic and bactericidal function of granulocytes obtained.

Honors and Awards: None

Publications: None

Serial No. NCI-6250

1. Baltimore Cancer Research Center
2. Research Pathology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Ultrastructure Studies of Acute Leukemia, Before and During Chemotherapy

Previous Serial Number: 6250

Principal Investigator: F. T. Sanel

Other Investigators: A.A. Serpick

Cooperating Units: None

Man Years:

Total:	1/4
Professional:	1/4
Other:	1/2 (see 6272)

Project Description:

Objectives:

These continuing studies are directed towards determining the responsiveness to, and cytological aspects of, phagocytosis provoking agents on peripheral blood and bone marrow cells from patients with acute leukemia before and during treatment. Concommitant search for virus is pursued.

Major Findings:

Monocytic cells from patients with acute monocytic and monomyelocytic leukemia exhibited vigorous responsive activity at surface membranes and ingested particles in phagosomes and secondary lysosomes. Specialized zipper-like complexes were elicited in about 20% of cases studied. There occurred where a) adjoining cells made contact by complicated interdigitation of the plasmalemma b) in areas where adjacent pseudopodia on the same cells approximated c) in cortical areas of the cytoplasm.

Significance to Cancer Research:

Formation of the elicited junctional complexes is undoubtedly related to the nature of the plasma membrane or its surface coat. The nature and relationship of these alterations to transformation from normal to leukemic cells may

be relevant to etiology, diagnosis and therapy.

Proposed Course:

1. Continued study of monocytic and monomyelocytic leukemias with phagocytosis provoking agents.
2. Cytochemical study of the glycocalyx utilizing lanthanum nitrate, ruthenium red and acid thorium dioxide.
3. Continued search for virus.

Publications:

Sanel, F.T. and Serpick, A.A.: Plasmalemmal and Subsurface Complexes in Human Leukemic Cells: Membrane Bonding by Zipperlike Junctions. Science 168:1458, 1970.

Serial No. NCI-6272

1. Baltimore Cancer Research Center
2. Research Pathology Section
3. Baltimore, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies of Myelomonocytic Leukemia in an Animal Model System

Previous Serial Number: None

Principal Investigator: F. T. Sanel

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4 (see 6250)

Project Description:

Objectives:

To study in Balb/c mice etiology, passaging under varied conditions, and ultrastructure of transplantable, lysozyme producing monomyelocytic WEHI-3 tumor cells, utilizing techniques of short term tissue culture, freeze-etch and conventional electron microscopy and histochemistry.

Major Findings:

"C" type immature and mature particles of the MLV prototype were identified in WEHI-3 tumors and invasive tumor cells in liver, spleen and bone marrow. Budding particles were observed at the plasmalemma and within cisternae of the endoplasmic reticulum. Lysozyme levels in urine, serum and tumor homogenates continue to be elevated in successive passages.

Significance to Cancer Research:

This animal system could be a valuable model for the study of etiology, diagnosis and therapy of human leukemias associated with elevated lysozyme levels.

Proposed Course:

1. Study of oncogenic potential of the "C" type particles.

2. Cytological localization of lysozyme.
3. Therapeutic studies.

Publications:

Sanel, F. T.: Virus Producing Cells in WEHI-3 Myelomonocytic Tumors. Proc. Amer. Assoc. for Cancer Research, 12:76, 1971. Abstract No. 301.

SUMMARY REPORT

DRUG RESEARCH AND DEVELOPMENT
(CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER)

CHEMOTHERAPY
NATIONAL CANCER INSTITUTE

July 1, 1970 - June 30, 1971

Introduction

The official name of the organization previously known as Cancer Chemotherapy National Service Center has now been changed to Drug Research and Development (DR&D), in order to reflect more accurately its true functions. Because of the recency of this change, most of the reports contained herein still use the term Cancer Chemotherapy National Service Center.

During the past year, the Drug Research and Development area continued to pursue aggressively its primary task of searching for and developing new and potentially more effective antitumor agents, as well as to develop new and better methods of drug selection and prediction of optimal drug schedules. With respect to the linear array, the organization is responsible for all pre-clinical work in the development of new drugs except that concerned with pre-clinical toxicology.

On July 1, 1970 the Program Analysis Branch was transferred back to DR&D, of which it had been a part until 1965. Thus the additional functions related to Data Processing, Literature Research, Publications and Scientific Records were added to the total responsibility of DR&D. The data processing and scientific records activities relate primarily to functions of the Drug Development and Drug Evaluation Branches of DR&D, but the literature and publications activities serve the entire Chemotherapy program.

The DR&D program is carried out under contracts with pharmaceutical and chemical companies, research institutes and academic institutions; through the laboratory operations carried out within a contract at Microbiological Associates, Inc. under direct staff supervision and collaboration; and in the in-house laboratory and other operations in Building 37. The activities depend heavily on additional collaboration with research grantees, other independent investigators throughout the world, and hundreds of industrial companies not involved in contracts, and especially on the invaluable collaboration with staff in other portions of the Chemotherapy Program and other areas of the National Cancer Institute.

The Drug Research and Development Program, involving about $16,000,000 in contract activities plus direct operations, is concerned primarily with the following specific areas:

1. Synthesis of new chemical agents.
2. Procurement and resynthesis of bulk chemicals and drugs for preclinical and clinical evaluation.
3. Development of new agents from fermentation, plant and other natural products.
4. Development and production of clinical dose forms for clinical trial.
5. Quality control for bulk and formulated drugs.
6. Primary screening and evaluation of new materials.
7. Bioassays, both in vivo and in vitro, to monitor the purification of fermentation and other natural products.
8. Study of optimal drug routes and regimens, including combination therapy and other means for improving drug effectiveness.
9. Development of new and improved laboratory methods for discovering, evaluating and utilizing potential antitumor agents.
10. Investigation of mechanisms of action and resistance of drugs of clinical interest.
11. Animal procurement, distribution and disease control.
12. Procurement of radiolabeled drugs for clinical pharmacologic and experimental studies.
13. Development and operation of data processing systems for both biological and chemical information.
14. Search, retrieval and dissemination of pertinent information from the world's literature on drugs and other aspects of cancer therapy.
15. Publication of the abstract journal, Cancer Chemotherapy Abstracts.
16. Publication of the scientific journal, Cancer Chemotherapy Reports.

Staff and Organization

The DR&D laboratory and contract activities during FY 1971 were carried out by a full and part-time staff of 107: 22 senior professionals (GS-13 or higher), 27 junior professionals, 6 guest workers and fellows, 13 laboratory technicians and support personnel, and 39 clerical and secretarial personnel. The present organization is presented below.

Drug Research and Development

Associate Scientific Director for Drug Research & Development - Saul A. Schepartz

Associate Chief for Laboratory Research - Abraham Goldin
Mammalian Genetics and Animal Production Section - Samuel M. Poiley, Head

Drug Development Branch - Harry B. Wood, Jr., Chief

Chemical and Drug Procurement Section - Robert R. Engle, Head
Chemical and Drug Records Section - Robert B. Ing, Head
Clinical Drug Distribution Section - J. Paul Davignon, Head
Natural Products Section - Jonathan L. Hartwell, Head

Drug Evaluation Branch - John M. Venditti, Chief

Biochemistry Section - Florence R. White, Head
Immunity & Immune Response Modifiers Section - John P. Glynn, Head
Screening Section - Betty J. Abbott, Head

Program Analysis Branch - Barbara R. Murray, Chief

Literature Research Section - Dorothy Algire, Head
Publications Section - William T. Soper, Head
Section on Data Processing and Computer Systems Development - Sidney Richman, Head
Scientific Records Section - George F. Hazard, Acting Head

Major Accomplishments

Selection of Agents for Screening - Stage I

During the past year continued emphasis has been placed on the pursuit of structure-activity relationships. A study was completed for the methanesulfonates and continued in the area of the quinones. In addition, multiple regression analyses were utilized, particularly in the area of nitrosoureas, in order to attempt a more rational design of better agents.

Major efforts were initiated toward the mechanization of chemical information. During the past year a computerized sub-structure search system was implemented, as was a computerized method of searching for duplicate compounds. Current efforts are directed toward the mechanization of most of the manual chemical information handling systems that have been employed since the beginning of the program. A total mechanized system should be implemented within the next 2-3 years.

During the period of this report, materials continued to be obtained through a variety of mechanisms, including direct purchase, synthesis contracts, natural product development contracts and the voluntary submission of a wide variety of materials by grantees and other investigators throughout the world. Listed below are the numbers of materials obtained during calendar year 1970 compared with comparable figures for the four prior years. In order to provide exact numbers for this report, it is necessary to use the calendar year rather than the fiscal year.

Materials Obtained

	1970	1969	1968	1967	1966
Synthetics and Crystalline Natural Products	8,521	9,820	10,915	7,413	8,008
Crude Fermentation Products	2,172	2,184	4,256	4,982	4,284
Crude Plant Products	3,499	5,109)	4,245)	6,253)	3,620
Animal Products	587	757)	641)	446)	
Total	14,779	17,870	20,057	19,094	15,912

The decrease in input since 1968 is due to three major factors: (1) the lack of staff, time and funds to visit various institutions and collect synthetic compounds; (2) the phase-out of the contract with Chas. Pfizer and Company, which brought about a major decrease in crude fermentation products as well as a small decrease in plant products; and (3) the general limitation of funds, inflation and the need to recollect active materials, which cut significantly into the procurement of new plant products.

There is a continued recognition of the importance of procurement and evaluation of new types of chemical structures. In this regard, it is worthwhile pointing out that the successful acquisition of new chemical agents from a variety of sources has been directly proportional to the number of visits made by DR&D staff to the collaborating institutions in order to discuss our program and actually collect new materials. Such visits have been made primarily to academic institutions in the past, but it is clear that visits to chemical and pharmaceutical companies, both domestic and foreign, will add immeasurably to the input of new materials to the program.

Determination of Antitumor Activity of New Agents - Stage II

For the past year or more the primary screen has consisted of the L1210 tumor, as well as the P388 leukemia for natural products. Although some natural products previously selected by the Walker 256 screen were still in process during the year, the effort with that tumor will be essentially phased out by July 1971. The shifts in screening emphasis are evident from the following tabulation of tests carried out during calendar year 1970 as compared with the testing levels for 1968 and 1969.

Screening Tests

	1970	1969	1968
L1210 Lymphoid Leukemia	79,892	80,545	107,445
P388 Lymphocytic Leukemia	42,270	43,223	14,903
Walker Carcinosarcoma 256	10,247	20,328	44,563
B16 Melanoma	1,342	1,240	286
Lewis Lung Carcinoma	3,033	925	244
Other Tumors	504	426	774
Total In Vivo	137,288	146,687	168,215

The P388 leukemia has proven to be exceedingly useful in detecting active natural products. As mentioned in last year's report, it is hoped that many of these materials will also possess L1210 activity when purified. A material with P388 activity alone, however, has already been selected for preclinical toxicology (Harringtonine, NSC-124147). Once a number of such agents have been evaluated, it may be possible to determine the role of P388 itself in selecting effective agents. An increase in the number of new active crude materials, because of the use of the P388 screen, has already occurred.

During calendar year 1970, 135 synthetic compounds and purified natural products showed confirmed activity (passed Stage II) and thus became candidates for the intensive evaluation needed to make the decision (Decision Point IIA) whether they are qualified to progress to dose formulation, toxicology and eventual clinical trial. This number may be contrasted with the 250 such materials detected during calendar year 1968 and 166 in 1969. The significant decrease is due to the elimination of the Walker 256 screen, and the overall decrease in input. The decision to eliminate the Walker 256 screen was, of course, based on several scientific bases, and a decrease in yield was predicted.

During calendar year 1970, 7 materials passed Decision Network IIA. (These are listed in tables in the reports of the Drug Development and Drug Evaluation Branches and need not be repeated here.) Five IND applications were filed during calendar year 1970. The materials reaching that stage were:

NSC-1895	Guanazole
NSC-71261	Beta-TGDR
NSC-107,392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone
NSC-123,127	Adriamycin
NSC-125,066	Bleomycin

Although the number of IND applications filed in 1970 is the same as the number filed in 1968 and 1969, the decreased numbers at earlier stages will be reflected in a decrease in the yield of new IND's within the next year or two.

The addition of a slow-growing tumor (Lewis Lung and/or B16) to the routine screen is being implemented. The kinetic characteristics of these tumors are being analyzed and we hope to have sufficient data within the next few months to begin utilizing them on a larger scale. Unless preliminary estimates are inaccurate, however, it appears that the yield from these tumors will be relatively small. That, together with the low yield of materials active in L1210 will result in an overall yield of new materials below the present one in 3000-4000. Thus it is essential to provide for a continued higher level of input.

Dependency Schedules - Stage IIIB

Major efforts have continued on the evaluation of new and old drugs in relation to optimal dosage schedules and routes of administration. The information generated in these studies has been utilized by the toxicologists and clinicians in the establishment of appropriate protocols for the further evaluation of these materials. Such studies have been of additional value in actually detecting compounds that may have otherwise been overlooked. An example is Emetine, hydrochloride, which had demonstrated rather marginal activity in L1210 several years ago. In current studies, significant activity was observed in the P388 leukemia at dosage schedules other than those used routinely. When tested under similar schedules in L1210, the drug demonstrated far greater activity than had been previously observed and has since passed Decision Networks II and III and entered clinical trial.

Therapeutic Trials in Animals - Stage IVB

Research efforts continued during the past year on the establishment of appropriate experimental designs for developing systematic trials of drug combinations in animals. The AKR spontaneous lymphoma has been the subject of intensive study and as a result of the encouraging results obtained thus far, a substantial production effort was undertaken and has resulted in the availability of large numbers of animals to carry out significant therapeutic studies.

Direct Laboratory Operations

During the past year laboratory studies were carried out in Building 37 in all areas of DR&D, with the exception of experimental dose formulation, since the laboratory has not yet been completed. The advantage of having resources available to expedite urgently needed studies expeditiously has already been adequately demonstrated. The laboratories in Building 37 have become an integral part of the overall program and will play an important role in future years.

Future Plans

Specific plans for a more aggressive effort on the part of DR&D in the acquisition and development of new drugs are currently being formulated. In line with projected decisions that new drugs must be provided for clinical study at a greater rate than they have been in the recent past, and that a more intensive effort needs to be applied to the discovery of effective drugs for the solid tumors of man such as breast, lung, and colon, significant changes in program operation will be required. As an initial step it is suggested that the input of new synthetic compounds and natural products for screening be approximately doubled. Secondly, as many as possible of the new materials should be tested not only in L1210 and P388, but also in one or more slow growing tumors such as the Lewis Lung Carcinoma and B16 Melanoma.

Substantial changes in the operations of the Drug Development, Drug Evaluation, and Program Analysis Branches will be required by this increased effort. It is likely that a series of prime contracts, each responsible for a portion of the total operation, will be initiated during the next year. Under a prime contractor system, a single contractor would be responsible, for example, for all of the routine screening contracts and the data derived therefrom. This prime contractor would obtain, analyze, summarize, and present to NCI all of the information needed for final decisions regarding the progression of the material from Decision Point IIA toward clinical trial.

Since an increase in total input and screening would ultimately result in increased levels of chemical production and other aspects of the entire program, they must also undergo expansions and operational changes in future years. Considerable thought and planning has already been given to the problems of an expanded program of this nature, and it is hoped that these changes can be accomplished with minimal stress.

During the next several years renewed emphasis will be placed on the development and evaluation of new screening methods, primarily methods that might select materials active in ways other than as inhibitors of nucleic acid synthesis. Thus, it is planned to investigate biochemical methods for evaluating new agents, and to study some of the basic mechanisms that cause a cell to enter the mitotic cycle in order to determine means for designing drugs that might either "turn on" or "turn off" a cell.

Emphasis in the synthetic area will be placed on the maximum exploitation of structure-activity relationships to the extent that the state of the art will permit, and on the search for new types of effective agents. Natural product procurement will be concentrated on higher plants plus a more systematic exploration of insects, marine organisms, and other sources of natural products.

An expanded program, as outlined above, will create tremendous pressure on our present communication methods. Considerable thought and effort will be devoted to the development and utilization of better alerting systems to detect new information worthy of rapid exploitation and general communication methods to provide effective interplay among all participants in the program and others in the field.

Serial No. NCI 3575

1. Office of Associate Scientific Director and Chief, CCNSC
2. --
3. NCI - Building 37 and Kenwood Laboratories Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemotherapy research including fundamental biological and biochemical investigations of antitumor agents

Previous Serial Number: Same

Principal Investigator: Abraham Goldin

Co-Principal Investigators: A. W. Schrecker, J. M. Venditti

Other Investigators: M. Straus, R. Kojima, A. Nicolin

Cooperating Units: M. Friedkin and N. Kaplan, University of California, San Diego; F. M. Huennekens, Scripps Clinic and Research Foundation; I. Wodinsky and C. Kensler, A. D. Little, Inc.; G. Cudkowicz, State University of New York at Buffalo; J. M. Venditti and staff, Drug Evaluation Branch, NCI; H. B. Wood, Jr. and R. R. Engle, Drug Development Branch, NCI; N. Mantel, Biometry Branch, NCI; S. Shackney, Laboratory of Chemical Pharmacology, NCI; J.A.R. Mead, Experimental Therapeutics, NCI; R. Gallo, Human Tumor Cell Biology Branch, NCI; E. Henderson and P. Carbone, Medicine Branch, NCI; I. Kline, S. Vadlamudi, and V. Waravdekar, Microbiological Associates, Inc., A. DiMarco, Farmitalia, Milan, Italy; E. Frei, III, E. Freireich and T. L. Loo, M. D. Anderson Hospital and Tumor Institute; G. Timmis, Allen & Hanburys Limited, Ware Hertfordshire, England; L. Israel, Centre Hospitalier Universitaire Lariboisiere, Paris, France; E. Bonmassar, University of Milan, Italy; S. Garattini, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy.

Man Years

Total: 8
Professional: 4-1/3
Others: 3-2/3 (plus animal care support from Mammalian Genetics and Animal Production Section, CCNSC)

Project Description:

Objectives:

To conduct fundamental investigations of potential and established clinical cancer chemotherapeutic agents; to obtain biological and biochemical knowledge concerning chemical agents capable of affecting malignant growth; to determine means for improvement of therapy with existing drugs; to investigate the influence of structural modification of drugs on the mechanism of antitumor action; to provide basic information of pertinence to the clinical use of such agents; to study specific problems encountered during the use of these agents in the clinic.

Specific objectives include:

I. Investigations of primary and secondary screening procedures and methodology employed in drug evaluation.

   A. Investigation of characteristics of ongoing screens and drug evaluation systems and of new test systems which may broaden the base of predictability of drugs for clinical activity. Emphasis is placed on predictability systems for clinically useful compounds which were not predicted by the standard screen and systems for the selection of new and novel structures which may have potential in clinical chemotherapy.

II. Investigation of the influence of drugs on tumor cells.

   A. Detailed investigation of mode of action of drugs including purine and pyrimidine antagonists, folic acid antagonists, alkylating agents, natural products, etc.

      1. Determination of the extent and duration of drug action.

      2. Study of the influence of drug on the mitotic cycle.

      3. Study of the lethal action on tumor cells.

      4. Investigation of problems related to drug resistance.

      5. The influence of chemical agents on metastasis and tumor cell sequestration including intracranial involvement.

      6. Studies of drug transport.

      7. Investigation of the sites of action of drugs.

      8. Study of the influence of drugs on the hematopoietic and tissue components of the host in relation to antitumor activity.

      9. Inhibition studies of RNA-dependent DNA polymerase.

International Activities Including Surveillance for New Agents

An important function of this office involves the maintenance of close relationships with world-wide cancer chemotherapy programs with a view to (a) information exchange pertaining to latest scientific developments pertaining to chemotherapy, (b) for the opening of new avenues of procurement of synthetic agents and natural products for screening and (c) for the identification of new compounds of potential interest for chemotherapy.

In the above, close liaison is being maintained with investigators in leading institutions, including universities, medical schools and pharmaceutical houses in countries such as England, France, India, Germany, Japan, etc., in the exchange of information and compounds, and in collaborative investigations. Active contact continues to be maintained through the Chemotherapy Committee of the International Union Contra Cancrum, the International Agency for Research in Cancer of the World Health Organization, Lyon, France, and through the associations developed through the joint U.S.-Japan Cooperative Science Program, etc., as well as through international meetings, interchange visitations and correspondence.

As part of this liaison program Drs. A. Goldin and Harry B. Wood visited a number of pharmaceutical and chemical companies and other institutions in Europe. Joint visits were made to the following:

1. Rhone Poulenc Chemical and Pharmaceutical Company in France. The specific interest in this visit stems from the fact that the antitumor agent rubidomycin (now known to be synonymous with daunomycin) comes from this company.

2. Asta-Werke Aktiengesellschaft Pharmaceutical Manufacturing Company in Germany. This was a follow-up visit in exploration of the clinical and preclinical data on Iphosphamide (Z-4942-Asta; NSC-109724).

3. Deutsches Krebsforschungszentrum (German Cancer Research Center). This visit was primarily for discussion of new synthetic colchicine derivatives.

4. Institute for Experimental Toxicology and Chemotherapy of the German Cancer Research Center in Heidelberg, Germany. Discussions were held on the carcinogenic action of diethylnitrosoamines, triazines, and alkylating agents.

5. Farbwerke Hoechst Company in Frankfurt, Germany. Specific discussion was held on the potential antitumor activity of cyclic 3'5'-monophosphates of adenosine, uridine and inosine and on the action of Poly I-poly C against Friend leukemia.

6. Institute de Ricerche Farmacologiche "Mario Negri", Milan, Italy and the Pharmacology Institute of the University of Milan for discussion of collaborative programs in progress.

7. Farmitalia Pharmaceutical Company in Milan, Italy for discussion of daunomycin and adriamycin and derivatives.

B. Investigation of factors which may influence drug action in relation to improvement of therapy.

III. Study of the influence of drugs on tumor-host interactions.

A. Investigation of the basis for drug specificity.

1. Exploration of the influence of novel schedules. Investigation of the underlying basis for drug scheduling and combination chemotherapy.

2. Detailed studies in inhibition analysis.

a. Determination of metabolite-antimetabolite interrelationships for folic acid congeners, purine antagonists, etc.

b. Investigation of the inhibition of de novo synthesis, lethal synthesis, feedback inhibition, etc.

c. Investigation of collateral sensitivity as a phenomenon which may lead to improved therapy.

B. Exploration of means for augmentation of the host contribution to chemotherapy.

C. Chemotherapeutic investigations on tumor cell kinetics with a view to the total eradication of the tumor cell population in the host.

IV. Antiviral chemotherapy.

A. Study of the action of drugs on virus-induced tumors.

V. Exploration of new and potentially useful biological and biochemical methodology for prediction, evaluation and improvement of drug therapy.

This program is planned so that it will be highly responsive to the needs of the preclinical and clinical chemotherapy program of the Cancer Institute.

In addition to the laboratories in our new building, the Kenwood Laboratories of Microbiological Associates staffed by Microbiological Associates professional and technical personnel serve as a collaborative laboratory resource for the research staff of this program. The chemotherapy research program collaborates closely with the ongoing programs in drug evaluation, drug development, immunology, organic chemistry and pharmacology. The program serves as a focal point for the interdigitation of chemotherapy research including the CCNSC screening, developmental and basic chemotherapeutic investigations with collaborating CCNSC contractors, and clinical programs of NCI and other institutions. It maintains close relationships with medical schools, pharmaceutical houses, university and research institutes, both in the United States and abroad in areas pertinent to chemotherapy.

Methods Employed: Standard laboratory procedures were employed.

8. The Cancer Institute in Milan, Italy in order to obtain an overview of their chemotherapy program.

9. Gruppo Lepetit in Milan for discussion of the antitumor agent alanosine, and rifampicin and its analogues.

10. Sandoz Ltd. in Basel, Switzerland for discussion of podophyllotoxin derivatives.

11. Geigy Company in Basel, Switzerland for review of studies on the antiviral activity of trifluoro thymidine deoxyriboside, adamantanamine etc.

12. Hoffman-LaRoche in Basel, Switzerland for discussion of benzodiapine and other agents.

Specific discussion was held with these groups and with other contacts on the submittal of compounds for testing to the Cancer Chemotherapy National Service Center and for the extention of informal collaborative relationships.

In addition A. Goldin attended the Eighth International Congress of Biochemistry in Montreux and Interlaken, Switzerland and the First Congress of the European Association for Cancer Research in Brussels, Belgium. Drs. S. Carter and A. Goldin spoke at the First Congress of the European Association for Cancer Research and also at the Universite Jules Bordet at the Centre des Tumeurs de L'Universite Libre, Brussels. Drs. Carter and Goldin also attended the Preclinical and Clinical Pharmacology Cooperative Group Meeting of the European Organization for Research on the Treatment of Cancer. The meetings in Brussels provided a good review of the recent advances and approaches in European laboratories and clinics. A. Goldin also visited J. L. Amiel at the Institute of Cancerology and Immunology of the Gustave-Roussy Institute. This visit was prompted by our interest in ellipticine and in recent publications of this group on 9-methoxy-ellipticine lactate.

Largely as the result of this liaison program an increasing number of Japanese, European, English etc. pharmaceutical and chemical companies as well as individual investigators are sending in new chemicals for screening and evaluation. Confidence in our program is also reflected in the increasing interest in sending potentially active drugs and drugs which may have reached the clinic elsewhere, to our program at as early a stage as possible.

The following talks describing various aspects and results of our program were given abroad and in the United States during the year by A. Goldin:

1. Preclinical Testing of Anticancer Drugs. Joint symposium Sandoz, Ciba, Hoffman LaRoche and Geigy drug companies.

2. Preclinical Studies with New Antitumor Agents. First Congress of European Association for Cancer Research, Brussels, Belgium.

3. Screening and Drug Evaluation. Institute of Jules Bordet, Centre des Tumeurs de L'Universite Libre, Brussels, Belgium.

4. Methodology in Investigating New Drugs. Asta-Werke Aktiengesellschaft, Brackwede, Germany.

5. Prediction of Drugs for Human Cancer from Animal Studies. Buffalo Academy of Medicine.

6. Preclinical Selection of Anticancer Agents. University of Michigan.

7. Combination Chemotherapy with Folate Antagonists. New York Academy of Sciences.

8. Effect of Cytotoxic Drugs on Hematopoietic Cells. In Vivo Studies. Johns Hopkins University.

Major Findings:

I. The Relationship of Structure to Antitumor Activity

A. Retrospective Analyses

As part of the overall Chemotherapy Program, retrospective analyses have been continued on the structure-activity relationships of drugs of clinical interest. The publications in this area include:

1. Relationship of Structure to Antitumor Activity in Compounds Related to Folic Acid. J.A.R. Mead, H. B. Wood, Jr., and A. Goldin, 1968.

2. Relationship of Structure to Antitumor Activity for Purine and Pyrimidine Nucleosides. A. Goldin, H. B. Wood, Jr., and R. R. Engle, 1968.

3. Preclinical Investigation of Alkylating Agents in Cancer Chemotherapy. A. Goldin and H. B. Wood, Jr., 1969.

4. Methanesulfonates: The Relationship of Structure to Antitumor Activity. J. S. Sandberg, H. B. Wood, Jr., R. R. Engle, J. M. Venditti, and A. Goldin. Cancer Chemotherapy Reports, in press.

A study on benzoquinone derivatives is in progress. These studies are being conducted in collaboration with the Drug Development Branch and the Drug Evaluation Branch of CCNSC. They are providing important information which is serving as a guide for further synthesis and investigation of new chemical agents. The studies are also contributing important background information for the collaborative studies of the Drug Development Branch with Drs. C. Hansch and Nelson Smith of Pomona College on the correlation of molecular structure with effectiveness of cancer therapeutic agents. New drugs continue to be synthesized under the direction of the Drug Development Branch based on the results of these retrospective analyses.

B. Other Structure-Activity Studies

1. Antileukemic Activity of Dimethanesulfonates. (S. Vadlamudi, M. Padarathsingh, V. S. Waravdekar and A. Goldin)

Two dimethanesulfonate derivatives, 1-propanol, 3,3'-(methylimino)di-, dimethanesulfonate, hydrochloride (NSC-84641) and 1-propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride (NSC-102627) have been obtained from Japan. These are closely related dimethanesulfonates, and investigations were conducted to characterize their antileukemic (L1210) activity and to compare this activity with that of cyclophosphamide. Both of these compounds were active over a wide range of treatment schedules and approximately equal in effectiveness to cyclophosphamide. Some advantage in the use of NSC-102627 appeared to reside in the ability to employ a higher dosage of drug without encountering toxicity for the host.

Following daily treatment with both of the dimethanesulfonates in leukemic mice there was a reduction in the number of spleen cells and this was reflected in a decrease in colony-forming units and an increase in survival time in recipient animals. The reduction of spleen cells was more extensive in both leukemic and non-leukemic animals treated with the dimethanesulfonate derivative NSC-84641 suggesting that this drug was more cytotoxic for spleen cells than was NSC-102627. In contrast to previous findings with cyclophosphamide, the two dimethanesulfonates did not appear to exert preferential cytotoxic action for bone marrow cells as compared with spleen cells.

2. Studies on the Immunosuppressive Properties of Related Dimethanesulfonates. (S. Vadlamudi, V. S. Waravdekar and A. Goldin)

A number of chemical agents which exhibit antileukemic activity have been shown to interfere to some degree with the immunological response of the host. Since it was demonstrated that the two structurally related dimethanesulfonates obtained from Japan, 1-propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride (NSC-102627) and 1-propanol, 3,3'-(methyl-imino)di-, dimethanesulfonate, hydrochloride (NSC-84641) exert antileukemic activity in animal test systems and are of clinical interest, comparison of their immunosuppressive properties was undertaken.

Although these two dimethanesulfonates are closely related and are both effective against leukemia L1210 they differ in their immunosuppressant activity. NSC-84641 was markedly immunosuppressive, its activity approximating that of cyclophosphamide. It exerted strong immunosuppressive action when administered prior to or subsequent to antigenic stimulus and had a marked capacity for establishment and persistence of a chimeric state. NSC-102627 on the other hand was only mildly immunosuppressive. It showed no immunosuppressant activity when administered prior

to antigenic stimulus, but moderate action when administered following antigen. It had only mild and transient activity in the induction of a chimeric state. These data support the current preferential clinical interest in NSC-102627.

3. Structure Activity Relationships for Fluorouracil Derivatives. (V. Pujman, J. S. Sandberg and L. Howsden)

Fluoxidine (5-fluoro-4-pyrimidinol) synthesized in Czechoslovakia had been reported to be effective against sarcoma-180 and two leukemia systems (L VUFB and LA VUFB). Fluoxidine was effective in increasing the life span of mice bearing early ascitic L1210 leukemia when administered by the intraperitoneal or oral routes. In direct comparisons of fluoxidine and 5-fluorouracil both agents showed similar anti-L1210 activity. An L1210 variant resistant to 5-fluorouracil was also cross-resistant to fluoxidine.

II. Metabolite-Antimetabolite Relationships

A. Folic Acid Antagonists

Based on the retrospective analysis on the "Relationship of structure to antitumor activity in compounds related to folic acid" by J.A.R. Mead, H. B. Wood, Jr. and A. Goldin, collaborative studies have been continued on folic acid antagonists. This includes systematic synthesis, evaluation and study of series of new antifolates, relating structure to antitumor activity. The homofolate derivatives and halogenated derivatives of methotrexate continue to be of considerable interest and a number of them are more effective than methotrexate itself in increasing survival time of mice with methotrexate resistant or sensitive lines of leukemia L1210. A number of new compounds recently synthesized are currently being tested and it is considered that this program has a reasonable likelihood of being productive in terms of the introduction of new compounds and in providing basic information pertaining to structure activity relationships.

A large body of data is being accumulated on the utilization of folate antagonists in combination with other agents and a number of such combinations appear worthy of additional clinical attention. Such combinations include BCNU plus MTX, cytoxan plus MTX, 5-fluorouracil plus MTX, 5-fluorouracil deoxyriboside plus MTX, ara-C plus MTX, methyl-GAG plus MTX, actinomycin-D plus MTX, etc. Such combinations have shown therapeutic synergism in the L1210 system.

III. Mechanism of Action of Antitumor Agents

A. Inhibition of RNA-dependent DNA Polymerase. (A. W. Schrecker and R. Gallo)

A program has been initiated to study the inhibition by antileukemic and antiviral agents of the RNA-dependent DNA polymerase discovered in 1970 by Howard Temin and David Baltimore in RNA tumor viruses and

by Robert C. Gallo (Chemotherapy, NCI) and Sol Spiegelman in human leukemic cells. It is planned, in collaboration with Dr. Gallo, to study the inhibition kinetics of the viral enzyme, of the leukemic cell enzyme, and of the DNA-dependent DNA polymerase of normal cells, using a number of known and novel compounds. The primary purpose of this study is the development of new therapeutic agents and of new screening methods. In addition the project is expected to clarify the molecular biological mechanism of the antitumor specificity of compounds used in experimental and clinical chemotherapy.

In preliminary experiments, the action of cytosine arabinoside 5'-triphosphate (ara-CTP) on the viral DNA polymerase has been studied. It has previously been shown by S. S. Cohen and others that ara-CTP is a competitive inhibitor of the incorporation of dCTP into DNA catalyzed by calf thymus DNA polymerase. Preliminary experiments suggest that the kinetics of inhibition by ara-CTP of dCTP incorporation with the viral enzyme resembles that found with the "normal" polymerase. The affinity of ara-CTP to the viral enzyme appeared to be of the same order or somewhat lower than in the case of the calf thymus enzyme. A Lineweaver-Burk plot of dCTP incorporation in DNA in presence of a crude viral enzyme showed biphasic kinetics, suggesting possible allosteric regulation.

In view of Kornberg's finding that Escherichia coli DNA polymerase has only one binding site for the template and since synthetic polynucleotides are superior templates for the viral RNA-dependent DNA polymerase, the potential inhibition by poly I-poly C of the replication of viral RNA could be a reason for its antiviral and antileukemic effect in addition to the known stimulation of interferon synthesis. Poly I-poly C did inhibit incorporation of dTTP into DNA catalyzed by the viral polymerase in presence of the viral template. However, this inhibition was only minor except at concentrations much higher than antiviral or antitumor concentrations effective in cell culture systems.

B. Thymidylate Synthetase in Drug-Resistant Murine Leukemia. (A. W. Schrecker and J.A.R. Mead)

In leukemic cells and in bacteria resistant to methotrexate, dihydrofolate reductase levels are generally greatly increased. In the resistant bacterial cells, thymidylate synthetase is increased concomitantly, suggesting a close genetic linkage between the two enzymes and supporting the hypothesis that development of resistance is a mutational event. A project has been initiated, in collaboration with Dr. J.A.R. Mead, to survey a number of methotrexate-resistant sublines of leukemia L1210 for a possible parallelism between dihydrofolate reductase and thymidylate synthetase levels. Such a study may help to find out whether development of drug resistance in leukemic cells is caused by a mutational change. A new convenient and reliable radioisotope assay for thymidylate synthetase with thin-layer chromatographic separation of the reaction product has been developed for the purpose of this study.

IV. Drug Evaluation Systems

A. Analysis of Screening Systems

Despite some reasonable success in the selection of screening systems which select compounds with predictable activity for the clinic the screening programs employed in the Cancer Chemotherapy National Service Center are under continual review for purposes of improvement and broadening of the base of screening. Professor L. F. Larionov published a review of screening procedures employed in Russia (Cancer Chemotherapy Reports 54: 71-77, 1970 - Commentary - Methods of Screening Anticancer Drugs in the Soviet Union). This article as well as a previous article by A. Goldin, A. Serpick and N. Mantel (Cancer Chemotherapy Reports 50: 173-218, 1966) on experimental screening procedures and clinical predictability value have now been discussed by various investigators including M. A. Schneiderman and C. C. Stock (Cancer Chemotherapy Reports 54: October 1970). A "Letter to the Editor" was also written for Cancer Chemotherapy Reports 54: 294-298, 1970 by A. Goldin and S. K. Carter, Chemotherapy, National Cancer Institute, and N. Mantel, Biometry Branch, National Cancer Institute, presenting the current program and underlying principles pertaining to screening. Several points are pertinent for this report. Currently model screening systems are being investigated which will identify new and novel structures not identified by systems such as leukemia L1210 and which will also have a high likelihood of clinical activity. Particular emphasis is being placed in this direction in obtaining tumor systems which will identify drugs that are active against tumors with a low proliferative pool. In a screening system where there has been judicious choice of combinations of experimental tumor systems which are mutually exclusive in their selection of compounds for the clinic, it is possible to compensate effectively for the inability to conduct clinical testing of agents which are negative when screening is by a single tumor system.

In any screening system one does expect some level of false negatives and false positives. The listing of false positives for any particular screen may be a tenuous practice. Drugs selected by a very quantitative system such as leukemia L1210 may be only tentatively classified as false positives if because of poor absorption, systemic inactivation or limiting toxicity for the modality of therapy employed, etc. they have not received adequate trial. If toxicity prevents an adequate trial this may not necessarily indicate a failure of the selecting screen. Additional pharmacologic or biochemical investigation may reveal means for appropriate control of undesirable toxicity and permit adequate testing. An adequate trial requires that a drug be administered in the range of the maximum tolerated dose and on an adequate dosage schedule. An interesting example is provided by hydroxyurea which was active in the leukemia L1210 system but was initially considered as a false positive. With further study it has now demonstrated to have clinical activity. Thus, with an increase of knowledge of drug action whether through use of experimental systems or by use in the clinic, there is

improvement of the ability to evaluate the results of screens such as leukemia L1210. So-called false positives by that screen are turning out to be true positives, once we have learned more about drug effects and cell cycle kinetics, how to schedule drug therapy more effectively, how to monitor the patient's disease status during therapy and what supportive measures to apply if toxic manifestations appear.

Professor Larionov in his paper concludes with a suggestion for a special international meeting pertaining to possible "international unification of screening procedures". Continuing review of the status, applicability and successes of screening programs must logically undergo continuing analysis in line with this suggestion of Professor Larionov.

The manuscript "The Chemotherapy of Human and Animal Acute Leukemia" by A. Goldin, J. S. Sandberg, E. Henderson, J. W. Newman, E. Frei and J. F. Holland has now been completed and is in press in Cancer Chemotherapy Reports. It is considered that this analysis may help in the further correlation of animal and clinical studies in remission induction and maintenance, including aspects such as consolidation, intensification, reinduction, resistance, combination chemotherapy, etc.

Further to the analysis of screening systems, A. Goldin and S. K. Carter have written a review chapter for Cancer Medicine entitled "Screening and Evaluation of Antitumor Agents". This article serves as a current review of the status of screening and evaluation of antitumor agents in relation to both preclinical test systems and the clinic. The preclinical methodology encompasses in vitro and in vivo screening and evaluation systems including screening and experimental approaches, the predictability value of the test systems for the clinic and a detailed discussion of preclinical toxicological and pharmacological evaluation. Clinical evaluation encompasses a discussion of determination of clinical activity in Phase I and Phase II studies, correlations of experimental and clinical schedule dependency, combination chemotherapy and factors influencing drug effectiveness in preclinical test systems and in the clinic. In this chapter there is a listing of test data for four animal screens and pooled data for twenty of the most commonly used antineoplastic agents in seven signal clinical tumor systems. The analysis of this data again reveals how tenuous a practice it is to list false positives for any particular screen. It is clear that most of the drugs have not been fully tested against all seven of the signal tumors and that the definitive labeling of negativity would be hazardous in most instances. A discussion is also included on principles of chemotherapy and prospective considerations. In view of the current discussions pertaining to screening and drug evaluation, this article is considered timely. It may also serve as a basic reference for students and preclinical and clinical investigators in cancer chemotherapy.

B. Leukemia P1798 as a Possible Experimental Model for Remission Induction and Remission Maintenance for Acute Leukemia. (S. Vadlamudi, M. Padarathsingh, V. S. Waravdekar and A. Goldin)

A program has been in progress which has been designed to identify a model system involving a transplantable tumor for the study of remission induction and remission maintenance.

The combination of vincristine plus prednisone has demonstrated enhanced activity in remission induction for clinical acute leukemia but the L1210 system has failed to predict a therapeutic advantage for this combination. Following the examination of a number of tumor systems lymphosarcoma P1798 was selected as a potential experimental chemotherapy model for the study of remission induction and remission maintenance. This tumor system has been demonstrated to be sensitive to vincristine, prednisone, L-asparaginase, cytosine arabinoside, methotrexate and cyclophosphamide. On daily administration of vincristine and prednisone and on sequential administration in which vincristine was administered as a single dose and prednisone was administered daily the combination appeared to be more effective than the drugs given individually. The data to date indicate that the lymphosarcoma P1798 transplantable tumor system merits further investigation as an adjunct to leukemia L1210 for the development of a chemotherapy model for the study of remission induction and remission maintenance for clinical acute leukemia. The P1798 model system may effectively supplement the spontaneous AKR system in this regard.

C. Studies on Correlation of In Vitro and In Vivo Effectiveness of Drugs. (S. Vadlamudi, W. A. Thomas and A. Goldin)

A sensitive line of the murine lymphoma L5178Y and an L-asparaginase resistant variant L5178Y/R were cultured in vitro. The induced resistance appeared heritable and was evident after a series of passages in vitro or in vivo. Cell growth and tumorigenic characteristics of the resistant variant were similar to those of the parent line. Removal of L-asparagine from the tissue culture medium was detrimental to the growth of the sensitive cells (L5178Y) but did not interfere with the growth of the resistant cells (L5178Y/R). Both in vitro and in vivo L-asparaginase was inhibitory to the growth of the sensitive tumor cells but did not affect the growth of the resistant cells. Both tumor lines were equally sensitive to treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU; NSC-409962) in vitro and in vivo. These results indicate the desirability of additional study of the relation of in vitro response of sensitive and resistant tumor cells to in vivo drug effectiveness.

V. Chemotherapy Studies

A. Scheduling and Dosage of Drugs in Sequential Chemotherapy of Leukemia L1210. (M. Straus and A. Goldin)

Further studies were conducted in a program designed to investigate new and novel dosage and scheduling effects with individual and combinations of drugs. In studies with methotrexate in the treatment of leukemia L1210 it was previously demonstrated that treatment with an initial relatively high dose, followed by lower doses, yielded survival times of the leukemic mice which were longer than those observed when constant dose levels were employed.

In a study where a series of doses of methotrexate was given to mice with leukemia L1210 and a single high dose of cytoxan was substituted for the MTX at varying times, it was determined that the greatest therapeutic advantage was obtained when the cytoxan was administered early in the therapeutic regimen.

It would appear that early administration of a high priming dose of cytoxan or methotrexate lowers the tumor cell population so that scheduled doses of MTX may kill tumor cells more effectively.

B. The Influence of Schedule of Administration of Mitomycin C in the Treatment of Leukemia L1210. (R. Kojima, A. Goldin and N. Mantel)

Studies were conducted on the schedule dependency characteristics of mitomycin C in the treatment of mice with leukemia L1210. It was observed that on standard schedules of administration of the drug there was only limited schedule dependency. Mitomycin C was approximately equally effective in increasing the survival time of leukemic mice on a daily, every 2, every 3, or every 4 day schedule. However on an every 7 or every 8 day schedule, there was a definite reduction in therapeutic effectiveness. The schedule dependency characteristics become more manifest on further alteration of scheduling. With the employment of a priming dose of mitomycin C followed by an intermittent schedule of therapy with low doses of the drug, improved therapeutic efficacy was noted. The priming dose of mitomycin C would appear to lower the leukemic cell population. The subsequent reduction in dose and the use of an intermittent schedule may in turn permit preferential recovery of the host.

C. Relationship of Dose Schedules to the Effectiveness of Adjuvant Chemotherapy. (S. R. Humphreys and K. Karrer)

The Lewis lung tumor model system in which the tumor is implanted to the hind leg of BDF_1 mice was employed in this study. Under these circumstances the tumor metastasizes preferentially to the lung and forms a primary tumor at the site of implantation. The prognosis is determined by the extent of metastases that develop, primarily in the lungs, early in the course of the disease.

In this study the tumorous leg was amputated at different times, treatment was initiated at different times following surgical amputation and the numbers of treatments, schedules of treatment and dosages were varied. No optimal single dose schedule could be identified. Treatment with cytoxan initiated following surgical

amputation indicated that each stage of the disease required a different optimal dose and dose schedule and that the optimal dose schedules became increasingly less effective as the disease progressed. The results of the experiments did suggest however that adjuvant chemotherapy with 2-3 doses at a level just under the limits of toxicity offered the largest range of effectiveness. Translated to possible clinical utilization of cyclophosphamide for the prevention of establishment and growth of micrometastases following surgery, it is suggested that this "prophylactic" treatment should be accomplished with sufficiently high and repeated dose levels. Insufficiently high dose levels could have no effect on inhibition of tumor growth but could be dangerous in that they may decrease the "host defenses" against tumor growth.

D. Combination Chemotherapy with L-asparaginase and Methotrexate. (S. Vadlamudi, M. Padarathsingh, V. S. Waravdekar and A. Goldin)

Since resistance of leukemia L5178Y to L-asparaginase occurs very rapidly, it was considered desirable to determine the potential of L-asparaginase when employed in combination with other antitumor agents. In the current study the combination of single treatment with L-asparaginase plus daily treatment with methotrexate demonstrated therapeutic synergism in that it resulted in a greater increase in the survival time of the leukemic mice than was observed on treatment with the individual drugs. The therapeutic advantage of the combination of L-asparaginase plus MTX was observed when treatment with L-asparaginase was administered on the morning of days 3, 7 or 14 following leukemic inoculation and methotrexate treatment was started on the afternoon of the same day. It is of interest that in studies conducted elsewhere in which the methotrexate was administered at less than three hours following L-asparaginase there was an actual loss in methotrexate effectiveness rather than a demonstration of synergism. The studies suggest that the demonstration of synergism with a combination of drugs may be markedly affected by the temporal relationships in administration of the drugs.

E. Effect of Concurrent Treatment with Terramycin and Streptonigrin on Rauscher Virus in BALB/c Mice. (S. Vadlamudi, J. H. Choudhry and A. Goldin)

Studies were conducted with streptonigrin alone and in combination with terramycin to determine the effect of treatment in BALB/c mice infected with Rauscher virus. Bioassay results showed that in untreated infected control mice the Rauscher virus titer of spleen and plasma increased progressively, and the median survival time of such mice was 57 days. Four daily treatments with streptonigrin temporarily reduced the virus titer in the plasma and spleen and increased the median survival time of the mice by 42% over untreated controls. In mice treated for four days with the combination of streptonigrin and terramycin the virus titer was reduced significantly immediately following treatment and 6 out of 10 mice survived an 86-day observation period.

F. Immunity Plus Chemotherapy

1. Combination Treatment with Cyclophosphamide and Isogeneic or Allogeneic Spleen or Bone Marrow Cells. (S. Vadlamudi, M. Padarathsingh, E. Bonmassar and A. Goldin)

Additional data have been gathered on the efficacy of immunotherapy with cytoxan plus isogeneic or allogeneic spleen or bone marrow cells in eradicating leukemia L1210 in CDF_1 mice. Generalized leukemia was observed three days following intraperitoneal inoculation of leukemic (L1210) ascites cells in CDF_1 mice. On day 3 after tumor implantation residual viable leukemic cells were detected in the peritoneal cavity and spleen of leukemic mice 6 hours following treatment with a relatively high dose (180 mg/kg) of cyclophosphamide. Mice receiving weekly intraperitoneal injections of X-irradiated leukemic (L1210) cells for 6 weeks were resistant to a challenge of tumor cells. When incubated in vitro, spleen and bone marrow cells of immune mice were able to inactivate viable leukemic cells as evidenced by failure to tumor growth in mice inoculated with these cells. Leukemic mice injected with immune spleen or bone marrow cells from isogeneic mice following treatment with cyclophosphamide survived a 60-day observation period. In one such experiment 90-day survivors were able to resist reinoculation of tumor cells. Leukemic (DBA/2) mice inoculated with allogeneic spleen cells following cyclophosphamide treatment survived for longer periods than mice injected with isogeneic spleen cells. These experiments demonstrate the potential usefulness of immunotherapy with isogeneic or allogeneic lymphocytes, in eradicating the surviving leukemic cells following intensive drug therapy in acute leukemia.

2. Antileukemic Chemotherapy Combined with Allogeneic Spleen Cells Differing for Minor Histocompatibility. (E. Bonmassar, S. Vadlamudi, A. Bonmassar and A. Goldin)

The studies on the increased efficacy of antileukemic chemotherapy combined with allogeneic spleen cells differing with respect to minor histocompatibility loci has now been extended to DBA/2 Cr mice. Leukemia L1210 has been shown to be highly histocompatible with this subline of DBA/2. The studies show an improved therapeutic response in the DBA/2 Cr mice bearing leukemia L1210 via a genetically controlled graft-versus-host reaction with allogeneic spleen cells in animals immunosuppressed with cyclophosphamide and subsequently treated with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). The allogeneic spleen cells in this instance were taken from BALB/c donors which share the same H-2 locus with the recipient but differ in multiple histocompatibilities. Employing the plaque forming test it was indicated that the capability of immunosuppressed DBA/2 Cr mice to produce 19 S cytotoxic antibodies against a foreign antigen is restored after administration of isogeneic (DBA/2 Cr) or allogeneic BALB/c spleen cells. These experiments suggest that even in the absence of any demonstrable

tumor specific antigens it might be possible through the use of a limited genetically controlled graft-versus-host reaction to improve therapeutic response. Possible clinical application could have as a basis an accurate donor-recipient selection designed to obtain an attenuated graft-versus-host reaction to be associated with further chemotherapeutic treatment.

3. Immunological Alteration of Leukemic Cells In Vivo after Treatment with an Antitumor Drug. (E. Bonmassar, A. Bonmassar, S. Vadlamudi and A. Goldin)

Evidence has been obtained that the antigenicity of leukemic cells may be altered as the result of treatment with an antitumor agent. L1210 leukemia was transplanted serially in mice and treated with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC, NSC-45388). Following treatment over several generations a leukemic line emerged which grew slowly and a marked increase in survival time of untreated mice was observed. When mice bearing the slow-growing tumor were treated with DIC or immunosuppressed with cyclophosphamide, the animals succumbed earlier with generalized leukemia. Furthermore, the slow-growing line showed unusually high sensitivity to chemotherapeutic treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea. These observations were reproducible and slow-growing lines were established following treatment with NSC-45388 on four separate occasions. The observations suggest that the slow-growing lines acquired strong antigenicity both for the hybrid CDF_1 and parent DBA/2 hosts. It would appear that DIC treatment may have selected highly antigenic variants or induced somatic mutations resulting in the appearance of strong new transplantation antigen(s).

This type of directed antigenic alteration of leukemic cells resulting in increased sensitivity to a second drug (BCNU) may provide a basis for rational improvement of sequential chemotherapy, related to the phenomenon of collateral sensitivity.

4. Further Studies on Antigenic Changes in L1210 Leukemic Cells Induced In Vivo by Imidazole Carboxamide Derivatives. (E. Bonmassar, A. Nicolin, S. Vadlamudi, I. Kline and A. Goldin)

Since it was demonstrated that daily treatment with 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) produced antigenic alteration of L1210 leukemic cells additional studies were conducted with related compounds. It has now been demonstrated that antigenic changes were also observed in leukemia L1210 following treatment with 5-(3,3-dimethyl-1-triazeno)pyrazole-4-carboxamide (NSC-114924) or 3(or 5)-amino-4-pyrazole-carboxamide (NSC-1402) both chemically related to NSC-45388. With both compounds, after the fourth transplant generation, the median survival time of untreated control mice increased progressively and BCNU effectiveness was enhanced. These increases were reversed when the animals were immunosuppressed with cytoxan.

The imidazole carboxamide derivative NSC-1402 appeared to alter tumor cell antigenicity even though it was essentially ineffective as an antileukemic agent.

5. Further Investigation of Antigenic Properties Induced in L1210 Leukemic Cells by Antitumor Agents. (A. Nicolin, S. Vadlamudi, E. Bonmassar and A. Goldin)

Further studies were conducted to determine whether the observation of Bonmassar et al. that drug alteration of the antigenicity of the leukemic cells, accompanying the origin of drug resistance, was a generalized phenomenon. Twenty-two L1210 leukemic sublines resistant to a variety of antitumor drugs were investigated in CDF_1 female mice to observe whether antigenic alteration had occurred. For 19/22 of the sublines resistant to various drugs the untreated controls lived longer than the sensitive controls. The increased median survival time of the mice inoculated with the drug resistant sublines in some instances was partially reversed following immunosuppression with cyclophosphamide. In 19 out of 22 of the drug resistant sublines 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was markedly effective in increasing the median survival time over sensitive controls. The therapeutic advantage in the treatment of the resistant sublines with BCNU was largely reversed when the animals were immunosuppressed with cyclophosphamide prior to inoculation of leukemic cells. The findings suggest that the occurrence of antigenic alteration of leukemic cells may be a common occurrence accompanying the origin of resistance to chemotherapy. These observations lend further support to the possibility of employing this approach for the improvement of sequential chemotherapy.

VI. Collaborative Studies

Collaborative studies with the Drug Evaluation Branch and the Biometry Branch, NCI were continued. (J. M. Venditti and Staff, I. Kline, S. Vadlamudi and Staff; N. Mantel and A. Goldin)

These involve studies on schedules of drug administration, sequential and concomitant combination chemotherapy, studies on resistance and cross-resistance, etc.

Collaborative studies with the Drug Development Branch, NCI have been continued in the area of structure-activity analysis in chemotherapy. (H. B. Wood, R. R. Engle and J. Driscoll)

Collaborative studies with the Medicine Branch, NCI on preclinical investigation of specific problems relating to the usage of drugs in the clinic. (P. Carbone and E. Henderson)

Significance to Bio-Medical Research and the Program of the Institute

The chemotherapy research program is operated in direct relationship to the needs of the Chemotherapy Program, NCI. The investigations are conducted at the NCI and as part of a collaborative program at the Kenwood Laboratories. The program is conducted in close coordination with the activities of the Drug Evaluation Branch, the Drug Development Branch, the Laboratory of

Chemical Pharmacology, and the Medicine Branch of the Cancer Institute. The research is dovetailed with the screening program and contract activities of CCNSC.

New methodology is transmitted to the screening program and pertinent basic observations are transmitted for possible clinical application. New drugs of clinical interest are investigated with respect to optimal usage and mechanism of action and recommendations are made to the clinic. Problems arising in the use of chemotherapeutic agents are subjected to additional investigation.

Proposed Course of the Project

The various aspects of the program will be continued and appropriate modifications made in accordance with the laboratory findings and the clinical interests.

Further emphasis will be placed on detailed investigations with leukemia L1210, Lewis lung carcinoma and resistant tumors including characterization of drugs with respect to their action in the mitotic cycle of normal and neoplastic cells. Studies will be conducted on the influence of cell synchrony in chemotherapeutic effectiveness of drugs. Additional tumor screening systems will be investigated with particular reference to drugs of clinical interest which are not active in the L1210 leukemia or Walker carcinosarcoma systems. This includes tumor systems such as P-388, P-1534, P-1798, the LPC1 plasma cell system, transplantable melanoma, viral-induced leukemias and functional and spontaneous tumors.

Studies will be continued on means for estimation of numbers and location of residual sequestered tumor cells and of means for their eradication.

Immuno-chemotherapy studies will be continued. Studies on the influence of drugs in altering tumor cell antigenicity will be extended from the point of view of improvement of therapy. Studies on the potentiating effect of relatively non-specific weak graft-versus-host reactions will also be investigated further.

Detailed evaluation and investigation of drugs of potential clinical interest will be continued. Additional investigation will be conducted on scheduling procedures which will best permit recommendations for clinical use of new agents. New and novel scheduling methodology will be investigated. Studies will be continued to determine procedures for reducing drug toxicity and improving therapeutic effectiveness. This will include investigation of differential rates of recovery from drug toxicity of host tissues as compared with the tumor cell population. Studies with the spleen colony assay system will be continued as an ancillary index for the determination of optimal scheduling of therapy. Additional studies will be conducted on the maintenance of drug levels in the blood and transport, sites of action, etc. Biological and biochemical investigations will be continued to determine the characteristics and mechanism of action of antitumor agents. Attention will be focused on the phenomena of tumor invasiveness and resistance and potential chemotherapeutic approaches. Studies will be conducted on possible means for

creating differential deficiencies of essential metabolite in tumor cells as opposed to vital cells of the host. In collaboration with clinical investigators, biochemical investigations will be extended to human tissues of interest. In relation to clinical interest, emphasis will continue to be placed on studies of remission induction and remission maintenance including intensification, consolidation, reinduction, etc. This will include study of suitable systems, characterization of drug action and factors which may influence the effectiveness of drugs in various aspects of remission induction and remission maintenance. Studies with the P-1798 leukemia will be continued in this regard. In view of the strong clinical interest in multiple drug therapy emphasis will be placed on further investigations of combination chemotherapy with two or more drugs and on adjuvant chemotherapy.

Various facets of programs in progress and problems of particular clinical interest will be investigated. Increasing attention will be focused on preclinical investigations in liaison with clinical investigators.

Honors and Awards: None

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MAMMALIAN GENETICS AND ANIMAL PRODUCTION SECTION
OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR AND CHIEF, CCNSC

INTRODUCTION

The mission of the Mammalian Genetics and Animal Production Section during the year 1970 was concerned primarily with the provision of defined laboratory animals for the Chemotherapy Area, NCI, and, as available, for other program areas of the Institute. Secondary activities included service laboratories for monitoring animal quality and project-related research.

Animal production functions were correlated with the requirements suggested by laboratory program changes. The availability of a broad base of resources provided sufficient elasticity for rapid increases or decreases in the output from various colonies so that the testing and research laboratories achieved their objectives without difficulty.

As in previous years, the Section's activities included a continuation of germfree foundation colonies; colonies of animals with defined microflora; animals raised in controlled environments; genetic-production centers for the propagation of inbred animals; large-scale production colonies; disease diagnostic laboratories; histocompatibility studies for the genetic characterization of the various strains of inbred animals; study of the effects of various environments upon the microbial status of animals; development of standards for the breeding, care and maintenance of laboratory animals; conferences on laboratory animal medicine and animal use in biomedical research; professional and sub-professional training; and an accreditation program for suppliers of laboratory animals for the program areas of the NCI. Revisions to these activities during this period served to improve animal quality, strengthen controls for continuity of the genetic characteristics of the animals, and improve the overall efficiency of the contract-supported animal production colonies. The Section provided animal care services for ten mammalian species used for research in the Chemotherapy Area of Building 37.

The Section furnishes a variety of supporting services such as guidance and counseling for the development of animal colonies and the maintenance of research animals in laboratory environments; assistance for the diagnosis and control of animal diseases; design of animal facilities; information concerning the availability of and uses for animals; equipment design and its applications; and supplies information of general interest. Staff personnel function as members of local, regional and national committees for the development of standards; participate in conferences and symposia; serve as instructors in training programs; and as members of committees in scientific societies. Staff members participate in symposia, in training and educational programs, and publish the results of their investigations in the general field of laboratory animal care and maintenance.

Section animal supply services comprised eight mammalian species subdivided into 64 categories, classified genetically into outbred, inbred and hybrid groups. Breeding animals, with two exceptions, are furnished by Genetic

Production centers. Beagle producers and the contractor for F344 rats furnish breeding animals for their respective colonies. Genetically characterized animals are also furnished as hosts for tumors and for special research studies. Quality control, such as laboratory monitoring techniques, maintenance of desired environmental factors, and optimum husbandry practices, are concentrated at the centers since the animals in these sites are the basis for the overall program.

Section staffing for contract-funded operations consists of one senior staff member, two professional associates, and one animal contracts assistant. Staffing for inhouse animal care includes one animal technician supervisor and three animal technicians.

The senior staff member and the two professional associates served as Project Officers or Assistant Project Officers for the following contracts:

BDF_1 Hybrid Mouse Production	11
CDF_1 Hybrid Mouse Production	9
Inbred Mouse Production	5
Outbred Rat Production	1
BALB/c Production	1
Inbred Rat Production	1
Beagle Hound Production	3
Genetic-Production Centers	6
Germfree Inbred Rodent Production	2
Germfree Inbred Rat Production	1
Germfree Outbred Rat Production	1
Breeder Production Center for Hamsters	1
Disease Diagnosis and Research	3
Genetic Characterization of Hosts and Tumors	1
Outbred Hamster Production	2
Standards for Animal Care and Maintenance	1
Conferences on Laboratory Animal Medicine and the Uses of Animals in Biomedical Research	1
Animal Husbandry Guidance and Research, Development of Special Strains, and Research in Laboratory Animal Medicine	1
	51

The senior staff member also serves as Assistant Project Officer for animal husbandry practices, and supplies counseling for these activities in research laboratories.

ACCOMPLISHMENTS

Animal Production

During 1970, as in previous years, changes in laboratory animal requirements necessitated collateral revisions in production schedules. C57BL/6, DBA/2, F344 rats, AKR mice and BDF_1 hybrid usage increased significantly. Major reductions in use were confined principally to BALB/c, C3H/He and outbred

Sprague-Dawley descendant rats. Outbred rats were used only as donors for Walker 256, and the small number required for this purpose were maintained as an isolator colony.

Hamster usage during this period was comparatively nominal. As a result, those issued to laboratories were raised in genetic centers in closely controlled environments, with a distinct improvement in quality. The germfree hamster continues to remain elusive, but a new approach will be attempted during 1971.

Toxicology studies required a slight increase in beagle hounds. These were furnished without difficulty because market supplies were quite adequate. The beagle producers continue to improve their methods, and the quality of these animals was advanced.

Fixed-price contracts supported the production and supply of the following animals:

Mice, Inbred: DBA/2, C57BL/6 and AKR/Lw
Mice, Hybrid: BDF_1 and CDF_1
Rats, Inbred: Fischer 344
Dogs: Beagle Hound

The total number of animals furnished to NCI Program areas from all sources (Table I - Appendix) represents a decrease of approximately 65,000 during 1970 in comparison to 1969. This change is due to a reduction in the use rates of outbred rats and hamsters and the contraction of laboratory studies as an outcome of budgetary limitations. Funding restrictions also caused the postponement of projects designed to decrease our reliance upon the Jackson Laboratory for C57BL/6, DBA/2 and AKR/Lw mice. Genetic-production center colonies have been slightly expanded and several rodent breeding colonies have been activated. The latter colonies produced the major portion of AKR mice used during 1970.

Genetic Centers

The germfree Genetic Centers maintained animal colonies as gnotobiotes. Pedigreed foundation colonies of 12 strains of rats are propagated at Tulane Medical School, 11 strains of mice and 6 of rats with ARS/Sprague-Dawley, and 10 strains of mice and 7 of rats by the Charles River Breeding Laboratories. ARS/Sprague-Dawley and the Charles River Breeding Laboratories also maintain isolator-reared pedigreed expansion colonies with defined microflora. The expansion colonies furnish breeding animals for large-scale production and for special studies. The germfree colonies are essentially insurance against the loss of the production program due to epizootics. Clean animals for restarts are constantly available.

The staff assigned to the isolator colonies have become increasingly competent in manipulating this equipment without contamination. For example, a number of isolators at Tulane have been in continuous operation for three years without a "break", and many others being maintained in the other

centers have been in continuous operation for two years.

The following strains were maintained by the centers during 1970:

Inbred Mice: BALB/cAn, DBA/2, C3H/He, C57BL/6, C3H$_f$/He, A/He, PRI/Pl, AKR/Lw, CFW/Pl, NIH/Pl, CBA/Cr, BALB/cM, AL/N and SJL/J.

Inbred Rats: F344, August 28807, Copenhagen, ACI 9935, OM/N, S5B/Pl, Albany, Buffalo, ACP, M520, Yoshida 38366, NBR/Pl and W/L.

Outbred Rats: Cr:RAR (Sprague-Dawley descendants).

The Tulane colonies established and maintained seven of the inbred rat strains developed by Curtis and Dunning. This center serves as a repository or "back-up" for Dr. Dunning's colonies.

Genetic-Production Centers

These centers maintain foundation, expansion and production colonies for on-going laboratory projects. They supply animals for research and the breeding animals for large-scale production colonies. Breeding activities are performed in modified conventional environments. As requested, they produce special hybrids for short-term studies, and furnish the starts for breeding colonies in foreign countries. During 1970, animals were shipped to the Republic of the Philippines, India, Canada, Italy, Czechoslovakia and Spain. The majority of the breeding animals were shipped to Lucknow and Bombay, India, for the Chemotherapy studies performed under the auspices of the two PL-480 funded contracts with the Central Drug Research Institute and the Cancer Research Institute. The Section furnished informational assistance via correspondence.

The conventional genetic-production centers maintained the following stocks and strains during 1970:

Inbred Mice: BALB/cAn, BALB/cJ, DBA/2, DBA/2Pl, C57BL/6, C57BL/6Ka, I^s, BSVR, BRVS, BRVR, BSVS, CFW/Pl, C57L, NZB/Cr, NZW/Cr, SJL/J, C3H/He, SM/J, AKR/Lw, A/He, A/J, PRI/Pl, CBA/J, NIH/Pl, C3H$_f$/He, C57BL/10Sc, AL/N, STR/Cr, B10.A, B10p^d/Cz, B10.A(2R), B10.A(5R), C3H/Bi, and B10.129(5M).

Hybrid Mice: AKD2F$_1$, ALZBF$_1$, BAF$_1$, BDF$_1$, B6AKF$_1$, B6KC3$_f$F$_1$, B6C3HF$_1$, CBF$_1$, CDF$_1$, C3ZBF$_1$, (C3ZBF$_1$xNZB)F$_1$, D2AKF$_1$, D2ZBF$_1$, (D2ZBF$_1$xNZB)F$_1$, ZBZWF$_1$, (ZBZWF$_1$xNZB)F$_1$, ZWZBF$_1$, and (ZWZBF$_1$xNZB)F$_1$.

Outbred Mice: Swiss and Swiss-Webster

Inbred Rats: ACI, ACP, Albany, August 28807, BN/Cr, Buffalo, Copenhagen, Fischer 344, Long Evans, Marshall 520, NBR/Pl, OM/N, SD/M, SH/N, S5B/Pl, Wistar/Furth, Wistar/Lewis and Yoshida.

Outbred Rats: Cr:RAR(SD), Cr:MGAPS(OM) and Cr:MGAPS(Gunn).

Guinea Pigs: Strain 2, Strain 13.

Hamsters: Syrian (Mesocricetus auratus) and Chinese (Cricetulus griseus).

Jirds: Mongolian gerbils (Meriones unguiculatus)

Unusual: Agouti (Dasyprocta aguti)

Inhouse Animal Colony Activities

The move to Building 37 on the NIH campus provided opportunities for the Section staff to continue its involvement directly with animal colony manipulations. Space assigned to the Section in this building included an animal room and a laboratory. During this period, small colonies of animals difficult to raise were established in order to investigate reproductive performance and nutritional requirements. The following strains, which were imported and expanded during 1969, have now attained production levels.

Inbred Rats: /Cr, S5B/P1.

Inbred Mice: NZB, C57BL/6Ka.

During this period the Section continued its responsibility for the care of research animals used for investigations in the laboratories on the fifth floor of Building 37. With the appointment and training of the staff, routine activities have functioned satisfactorily.

Service Laboratories

The Battelle Memorial Institute (BMI), Illinois Institute of Technology Research Institute (IITRI), and New Jersey College of Medicine and Dentistry (NJCMD) functioned as service laboratories in the area of disease diagnosis and provided monitoring assistance for the upgrading of animal quality. BMI was primarily concerned with the routine culturing of fecal samples for the presence or absence of Salmonella spp. and Pseudomonas aeruginosa. Samples were received monthly from animal production colonies and research laboratories. Positive primary cultures were investigated in order to identify the serologic type. Unusual serotypes were submitted to the Enteric Diseases Section of the Ohio State Health Department Laboratories. Questionable cultures were submitted to the National Communicable Diseases Center for final confirmation. The number of positive samples continues to decline and, during this period, they comprised less than 1% of the total submitted. The species of salmonella detected during this period have not been identified as potentially pathogenic for laboratory animals with the exception of one group positive for S. typhimurium.

BMI, as time and funds permitted, cultured samples of food and bedding, and various material collected from the premises of infected colonies. The research portion of this contract constitutes approximately 10% of the total funding. Within this limited time, studies were continued to develop improved culturing techniques, a more sensitive detection system for P.

aeruginosa, and further explorations concerning the salmonella inhibitory substance produced by Aerobacter cloacae.

The IITRI laboratory furnished diagnostic services for the detection of Salmonella spp. and Pseudomonas aeruginosa, as scheduled, and a variety of other laboratory services. A significant portion of the contract is assigned to "trouble-shooting" activities, such as enzootic or epizootic conditions in production and research animal colonies. During 1970, such studies included conditions attributed to bacterial, viral or parasitic agents, or a combination of two or more of these factors. This group also continued its monitoring of animals maintained in germfree isolators. Staff members have competence in bacteriology, histopathology, virology and parasitology. With the advent of the agouti and the paucity of information concerning this species, it was felt that it would be desirable to acquire data concerning its physiologic parameters. These studies are continuing.

Since IITRI and BMI receive fecal samples from each supplier during alternate months, the former has been able to confirm BMI's observations that the presence of Salmonella spp. continues to decline. This fact is attributable to improvements in animal care methods and the availability of improved equipment.

NJCMD, during 1970, furnished vaccinia vaccine for the protection of mouse colonies against infectious Ectromelia and performed virus diagnostic services as scheduled. Due to budgetary and space limitations, the number of virus tests assigned to a particular source was dependent upon its importance in the overall program. Accredited, but non-contract suppliers, were assigned Ectromelia, Polyoma, and LCM; large-scale contract suppliers were assigned Ectromelia, Polyoma, LCM, MHV, and MadV; and Genetic Centers were assigned Ectromelia, Polyoma, LCM, MHV, MadV, Reo 3, Sendai, PVM, Theiler's (GDVII), Toolan's H-1, K, and SV5 viruses. During the latter part of this period, the profile was expanded with the inclusion of the Rauscher and Moloney viruses.

NJCMD furnished diagnostic assistance and vaccine for several epizootics of Ectromelia in institutions in the United States and one in a foreign country. Ectromelia has not been detected in the past in commercial colonies. Managers of commercial colonies are urged to vaccinate all imports and their offspring for a minimum of three generations, and continue to vaccinate breeding animals thereafter.

Virus testing of tumors has been continued. Depositors of contaminated tumors have been notified of the results of these tests, and infected tumors disposed of in accordance with their instructions. During 1970, the contractor performed 54,000 serum tests, tested 1,900 tumors, and supplied 50,000 ml of vaccine. Ectromelia services were again provided on a worldwide basis to the biomedical community because the frequent transfers of biological material, if unmonitored, could be the cause of panzootics.

Health Research, Inc. (HRI), continued its studies of the histocompatibility relationships among counterpart sublines of the mammalian species strains mentioned in the various centers. Skin graft studies were performed at

9-10 month intervals, and they incorporated reference animals from the NIH and the Jackson Laboratory. Special studies were undertaken as indicated; for example, to determine the histocompatibility relationship between hybrids and parental strains, or to identify the karyotype of an apparently aberrant tumor. Aberrant C3H/He sublines have been replaced, and F_1's were grafted, with satisfactory results.

The Mason Research Institute (MRI), under the terms of a contract supported by General Laboratories and Clinics, continued its studies of histocompatibility relationships between sublines of inbred rat strains. In addition, this contractor has been identifying the Hag antigens of these strains. The contractor has, from time to time, studied the skin graft index of outbred rat stocks, and the graft index of one strain grafted to another strain, both of which had an ancestor in common 20 or more generations in the past.

The incidence of dead and ill animals reported by consignees during 1970 was 2.56%, a decrease of 1.02% in comparison to 1969. This decrease is due to improvements in husbandry practices in production and research colonies, and revisions in packaging as a means to overcome exposure due to inclement weather and improper handling while the animals are in transit.

The Accreditation Program

The CCNSC program for the accreditation of suppliers or potential suppliers of animals for testing and research activities once again demonstrated its value as a segment of the quality control complex. A minimum of two inspection visits to each colony was made by Section staff members during 1970.

Inspection site visits were made to previously unaccredited organizations which had submitted resumes in response to sources sought notices posted in the Commerce Business Daily. The majority of these companies were accreditable and became recipients of RFP's. Several had been visited and, during the interim, had upgraded facilities and methods.

Many organizations, in anticipation of additional refinements in animal production activities, are beginning to revise physical plants and improve air handling systems. Unfortunately, several organizations deteriorated in quality, failed to cooperate with our laboratory monitoring activities, and were dropped from our listing of accredited potential sources.

In the course of accreditation visits, advice was furnished for colony management, building design, and the prevention of disease.

Task Order No. 6 of Contract No. PH43-64-44 produced the following standards:

Nomenclature System for Outbred Animals
Chickens - Standards and Guidelines
Standards for Procuring, Compounding, Holding and Transporting Non-Human Primates

These standards served to update the accreditation program and, as indicated,

were incorporated within the framework of animal production contracts.

Task Order No. 16 of Contract No. PH43-64-44 arranged conferences in the areas of Gnotobiotes, A Guide to Postdoctoral Education in Laboratory Animal Medicine, Nonhuman Primate Procurement, Animal Models for Biomedical Research III, and Nonhuman Primates. Proceedings of these conferences were published and made available to the scientific community.

During 1970, visits were made to production and research animal colonies as noted below:

Germfree Genetic Centers	19
Genetic-Production Centers	26
Rodent Production Centers	6
Beagle Production Colonies	6
Inbred Mouse Production	4
Inbred Rodent Colonies	87
Hybrid Mouse Production	32
Contract Supported Laboratories	48
Service Laboratories	17
Miscellaneous Visits	58
	303

Technical Assistance

Requests for advice and assistance by telephone and correspondence increased in comparison to those received in prior years. Inquiries included assistance for the alleviation of diseases; facilities design; sources of supply for species and strains of biological materials; care and breeding of laboratory animals; and references to reports of investigations which employed laboratory animals. Staff members continued to serve on national and regional committees.

The Section's exhibit and the Chemotherapy exhibit were presented by request during the meetings of the American Veterinary Medical Association and the American Association for Laboratory Animal Science.

PROPOSED COURSE OF PROJECT

Investigations directed towards improvements in animal quality will be continued. The successful outcome of preliminary studies involving the propagation of animal colonies in maximum containment environments will be expanded. Results to date indicate that healthy animals free of parasites can remain in this state when used for laboratory studies for a period of one year.

The 3-stage production of isolator-dependent colonies will be expanded. Section staff are convinced that this system is the route to follow in the future.

Laboratory projects for the future will include identification of physiologic and biochemical parameters for the various strains, genetic characterizations in addition to skin graft techniques, and improvements in animal husbandry practices.

Diagnostic programs will be expanded in order to include pathogenic organisms in addition to Salmonella spp. and P. Aeruginosa. Emphasis will be placed upon the monitoring of seemingly "normal" animals.

Zonal colony management systems investigations will be expanded with the expectation that these methods will eventually become routine for large-scale production colonies.

The extent of these investigations will be contingent upon availability of funds.

PUBLICATIONS

Poiley, S.M.: A survey of indigenous murine viruses in a variety of production and research animal facilities. Laboratory Animal Care, Vol. 20, No. 4, Part I, Aug. 1970, pp. 643-650.

Poiley, S.M., Chairman, Committee on Nomenclature of the Institute of Laboratory Animal Resources, Washington, D.C.: A nomenclatural system for outbred animals. Laboratory Animal Care, Vol. 20, No. 5, Oct. 1970, pp. 903-906.

TABLE I

ANIMALS SUPPLIED DURING CALENDAR YEARS 1969 and 1970

Inbred Mice	1970	1969	+ Increase - Decrease
A/He	10,236	10,372	- 136
A/J	1,883	1,442	+ 441
AKR/Lw	123,741	71,970	+ 51,771
-pregnants	4	10	- 6
-litters	--	30	- 30
AL/N	483	136	+ 347
A/St	--	50	- 50
BALB/cAn	153,050	219,610	- 66,560
-pregnants	10,501	4,879	+ 5,622
-litters	164	782	- 618
BALB/cJ	306	725	- 419
BALB/cM	--	336	- 336
B10.A	2,181	2,826	- 645
B10.129(5M)	1,799	3,577	- 1,778
B10.A(2R)	2,164	2,269	- 105
B10.A(5R)	2,281	1,090	+ 1,191
B10p^{d}/Cz	1,707	1,074	+ 633
B10/Cz	--	59	- 59
BSVS/Sr	4	--	+ 4
CBA/J	1,922	1,917	+ 5
-litters	--	2	- 2
CFW/Pl	171	163	+ 8
C3H/Bi	165	610	- 445
C3H/He	28,309	47,019	- 18,710
-pregnants	15	1,489	- 1,474
C3H$_{f}$/He	3,128	3,604	- 476
C57BL/6	91,623	70,290	+ 21,333
-pregnants	76	--	+ 76
-litters	5	10	- 5
C57BL/10Sc	3,201	1,876	+ 1,325
C57L	806	1,781	- 975
C57BL/6Ka	2,422	261	+ 2,161
-pregnants	21	6	+ 15
C58/J	--	55	- 55
DBA/1J	20	20	--
DBA/2	99,516	88,097	+ 11,419
-pregnants	6	--	+ 6
-litters	10	8	+ 2
I^{s}-pregnant	--	1	- 1
NIH/Pl	1,289	1,135	+ 154
-pregnants	30	103	- 73
NZB	930	325	+ 605
-pregnants	5	3	+ 2
-litters	1	5	- 4

TABLE I
(Continued)

Inbred Mice	1970	1969	+ Increase - Decrease	
NZW	1,786	1,073	+	713
-litters	1	6	-	5
PRI/Pl	2,419	3,138	-	719
SJL/J	5,552	1,283	+	4,269
SM/J	978	1,033	-	55
	544,072	539,216	+	4,856
Pregnants	10,658	6,491	+	4,167
Litters	181	843	-	662
Hybrid Mice				
$AKD2F_1$	2,336	1,387	+	949
$ALZBF_1$	44	5	+	39
BAF_1	4,604	4,257	+	347
BDF_1	1,259,877	1,219,191	+	40,686
-pregnants	6	--	+	6
$B6AKF_1$	2,364	4,999	-	2,635
$B6KC3_fF_1$	938	--	+	938
$B6C3HF_1$	800	--	+	800
CBF_1	7,698	7,411	+	287
CDF_1	286,744	289,468	-	2,724
-pregnants	2	--	+	2
-litters	--	10	-	10
$C3B10F_1$	--	2,181	-	2,181
$C3AKF_1$	--	198	-	198
$C3ZBF_1$	117	--	+	117
$(C3ZBF_1xNZB)F_1$	87	--	+	87
$D2AKF_1$	4,140	3,077	+	1,063
$D2ZBF_1$	65	--	+	65
$(D2ZBF_1xNZB)F_1$	103	--	+	103
$ZBZWF_1$	235	489	-	254
$(ZBZWF_1xNZB)F_1$	246	36	+	210
$ZWZBF_1$	962	1,078	-	116
-pregnants	358	306	+	52
$(ZWZBF_1xNZB)F_1$	10	--	+	10
	1,571,370	1,533,777	+	37,593
Pregnants	366	306	+	60
Litters	--	10	-	10
Outbred Mice				
Cr:MGAPS(S)	10,132	8,655	+	1,477
-pregnants	41	29	+	12
Cr:MGAPS(SW)	1,345	--	+	1,345
	11,477	8,655	+	2,822
Pregnants	41	29	+	12

TABLE I
(Continued)

Inbred Rats	1970	1969	+ Increase - Decrease
ACI 9935	2,494	1,702	+ 792
ACP	219	27	+ 192
Albany	521	299	+ 222
August 28807	978	878	+ 100
BN/Cr	315	7	+ 308
Buffalo	996	1,159	- 163
CAR/N	--	30	- 30
CAS/N	--	16	- 16
COP 2331	1,188	616	+ 572
Fischer 344	66,229	32,928	+ 33,301
-pregnants	3	--	+ 3
LE/Cr	48	--	+ 48
Marshall 520	562	567	- 5
NBR/Pl	4,138	1,110	+ 3,028
-pregnants	2	--	+ 2
OM/N	17	176	- 159
-pregnants	3	6	- 3
SD/M	1,289	376	+ 913
-litters	--	8	- 8
SH/N	317	33	+ 284
S5B/Pl	736	216	+ 520
W/Furth	4,592	355	+ 4,237
-pregnants	7	--	+ 7
W/Lewis	7,457	4,861	+ 2,596
-pregnants	19	--	+ 19
-litters	2	--	+ 2
Yoshida 38366	225	35	+ 190
	92,321	45,391	+ 46,930
Pregnants	34	6	+ 28
Litters	2	8	- 6
Outbred Rats			
Cr:MGAPS(Gunn)	40	76	- 36
Cr:RAR(SD)	66,967	220,625	- 153,658
-pregnants	88	47	+ 41
-litters	--	2	- 2
Holtzman	17	--	+ 17
Cr:MGAPS(OM)	1,057	--	+ 1,057
	68,081	220,701	- 152,620
Pregnants	88	47	+ 41
Litters	--	2	- 2
Hamsters			
Cr:RGH(SYR)	55,811	65,980	- 10,169
Pregnants	117	--	+ 117
Litters	--	2	- 2

TABLE I
(Continued)

	1970	1969	+ Increase - Decrease	
Dogs	401	205	+	196
Guinea Pigs	207	170	+	37
Gerbils				
Cr:MGAPS(MON)	320	--	+	320
GRAND TOTALS				
Mice	2,126,919	2,081,648	+	45,271
Pregnants	11,065	6,826	+	4,239
Litters	181	853	-	672
Inbred Rats	92,321	45,391	+	46,930
Pregnants	34	6	+	28
Litters	2	8	-	6
Outbred Rats	68,081	220,701	-	152,620
Pregnants	88	47	+	41
Litters	--	2	-	2
Hamsters	55,811	65,980	-	10,169
Pregnants	117	--	+	117
Litters	--	2	-	2
Guinea Pigs	207	170	+	37
Litters	--	2	-	2
Dogs	401	205	+	196
Gerbils	320	--	+	320
	2,344,060	2,414,095	-	70,035
Pregnants	11,304	6,879	+	4,425
Litters	183	867	-	684

ANNUAL REPORT

DRUG DEVELOPMENT BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

Objectives

The Drug Development Branch is engaged in a cooperative approach to the problems of cancer chemotherapy through the research and development of more effective chemical agents for use in humans. Past experience has clearly demonstrated that effective antitumor agents are not restricted to a single, or even a few, chemical classes but that the chemical-structural parameters are broad. One must conclude then that all effective agents are not operating by a single mechanism. Therefore, efforts are directed towards the chemical modifications of existing drugs to increase their activity and/or decrease their toxicity, and to search for new agents via chemical synthesis, or from natural sources. The latter category includes higher plants, marine invertebrates, insects, other animals, algae and fermentation filtrates. Materials from these sources will be evaluated in the primary screen and those agents demonstrating sufficient activity will be subjected to toxicological studies and possible clinical evaluation.

Research efforts are, in large measure, based on leads provided by the primary screens, clinical observations, biochemical and toxicological studies, literature reports, and the surveillance of the world-wide scientific community for information on new drugs. As the vast storehouse of information is accumulating and greater achievement being experienced in the control of the more rapidly proliferating phase of cell growth, the search for new structural designs aimed against the slower growing tumors and tumors of the central nervous system will be increased.

As part of the broad Chemotherapy Program the Drug Development Branch has three major objectives:

I. Fundamental Chemical Research aimed at supplying the program with new antitumor agents; this is carried out via:

   a. chemical synthesis of new compounds; and

   b. extraction, isolation and identification of new active agents from natural sources.

II. Applied Services:

   a. the chemical resynthesis and production of active agents;

   b. the quality control and analytical evaluation of drugs and bulk chemicals for clinical and preclinical toxicological evaluations, and x-ray structural analysis;

c. the development and production of suitable dose formulations for use in clinical studies;

d. the storage and distribution of bulk chemicals and clinical dose formulations;

e. the accessioning, chemical nomenclature, development, maintenance and utilization of a computer data bank for chemical and drug information of materials entering the program; and

f. the correlation of chemical structure to biological activity.

III. World-wide Surveillance of information and collection of chemicals and drugs for biological evaluation.

Implementation of Program

These objectives are carried out through the activities of the Branch's four Sections and its laboratory program which are:

Chemical and Drug Procurement Section. Objectives are the acquisition of new synthetic compounds for antitumor evaluation and the procurement of larger quantities for advanced screening, confirmational testing, pharmacological evaluation and clinical trial. The synthetic activity also provides new compounds for primary screening and the improvement of procedures for large scale production.

The chemical-preparative activities include the methodology and synthesis of bulk chemicals and drugs and the preparation of high purity radiolabeled materials for the Chemotherapy Program.

The quality control activities provide the analytical check on the identity, assay and purity of compounds and formulated drugs as well as the solubility and stability data on such materials.

Natural Products Section. Objective is the development of novel materials from: 1) higher plants and animal (marine and insect) products and 2) microbial and algal sources. In support of these tasks, the Section maintains a world-wide program (partly contract and partly non-contract) for the collection of natural products, the preparation of extracts, fractionation of active extracts and the isolation and identification of the active components; in collaboration with the Chemical and Drug Procurement Section, provides for the acquisition of large quantities of active materials needed for toxicological and clinical studies. Also, the Natural Products Section directs a program for the investigation of two groups of organisms, the actinomycetes and the fungi which have shown remarkable biosynthetic capabilities. This work requires development and chemical isolation. Surveillance of the literature is maintained as a means of obtaining new materials.

Clinical Drug Distribution Section. Objective is to provide the clinical program with formulated drugs in sufficient quantity and of uniform quality. Such activity includes the development of new dose forms for clinical investi-

gations, the surveillance of the quality of such dose forms and the maintenance of adequate supply and distribution of such drugs to the clinical investigators. In addition, the Section provides the necessary supportive data for the filing of IND applications with the Food and Drug Administration.

Chemical and Drug Records Section. Objectives are to 1) assist in the worldwide surveillance and collection of new drugs and provide the program with possible sources of such agents, 2) manage the acquisition of chemicals contributed to the Program, 3) provide systematic names for all compounds entering the Program, 4) insure the usage of proper nomenclature for all compounds and drugs in publications emanating from the Chemotherapy Program, 5) develop, maintain, and utilize a chemical data bank on materials of use to the program, 6) maintain adequate storage and inventory of bulk chemicals, and 7) coordinate the distribution of materials to screeners and other investigators. Surveillance is also provided over industrial "Commercial Discreet" products.

Laboratory Research on Chemical Drug Design. A laboratory activity is being developed to provide supportive basic chemical knowledge on drug design for the Chemotherapy Program via chemical synthesis of compounds of interest, the identification and preparation of drug metabolites, the determination of physical-chemical properties of active materials and research in new methodology of drug formulation.

Even though the laboratory is not fully operational and only partially staffed, research in the synthetic and instrumental areas is underway. Current Individual Progress Reports, Serial nos. NCI-3580 - 3581 are presented later in this report.

Branch Staff

The Drug Development Branch is composed of a staff of 31 persons consisting of six senior professional scientists (GS-13 and above), eight other professional staff members (GS-9 and above), two PHS Commissioned Corps Officers, one NIH Research Fellow, one Guest Worker, three Visiting Fellows, and the remainder technical clerical staff.

The Branch is divided into four Sections and those responsible for each activity are as follows:

Drug Development Branch
Chief -- Dr. Harry B. Wood, Jr.

Chemical and Drug Procurement Section
Head -- Dr. Robert R. Engle

Natural Products Section
Head -- Dr. Jonathan L. Hartwell

Clinical Drug Distribution Section
Head -- Mr. J. Paul Davignon

Chemical and Drug Records Section
Head -- Mr. Robert B. Ing

The Chemical Quality Control Unit (Head, Mr. C. A. Hewitt) operates as an activity under the Chemical and Drug Procurement Section. The Antibiotic Unit (Head, Dr. A. R. Stanley) operates as an activity under the Natural Products Section. The Chemical Laboratory Unit (Head, Dr. John S. Driscoll) operates as an activity under the Office of the Chief of the Branch.

Operation of Program

The methods for selecting new agents for input into the primary screen is gradually changing. More emphasis is being directed toward selectivity of agents and less emphasis towards random procurement. Random sampling of the chemical universe cannot be abandoned completely because it continues to enjoy success in providing active materials for the program. However, as the structure-activity mosaic gradually takes shape, the choice of synthetic compounds for introduction into the screen will emerge more from direct synthesis and will gradually replace random sample screening. The gradual mutation of randomization to directed selection of agents for screening will be generated from review and evaluation of chemical and biological data from within the program, in the literature and other related programs. Consideration is directed toward antitumor structure-activity relationships with emphasis on new and promising structural classes and de-emphasis or elimination of adequately studied classes or non-productive structural classes.

Since a substantial number of compounds are contributed to the Program on a voluntary basis, good public relations must be and have been maintained with the industrial firms, academic personnel and other governmental agencies by personal contact, by reporting of data and by making special handling of unusual agents a matter of normal service. With the increasing complexity of data processing and growth of the Program, constant awareness of the problems of "Commercial Discreet" data must be ever present. A total of 2045 individual suppliers have contributed samples to the Program representing industrial organizations, academic institutions and other government agencies. During 1970, there have been 142 new non-industrial suppliers and 47 new industrial suppliers submitting materials for primary screening. Even though the majority of these contributors are domestic suppliers, many materials have been supplied from foreign countries. The increasing number of contributors is an index of the enthusiasm for the Program by the scientific community.

New chemical structures or analogs, developed through biochemical, cell cycle kinetics studies, pharmacological and pharmaceutical rationales and biological activities demonstrated in non-cancer programs, should provide additional input to the directed synthesis approach. Chemical modification and structural analogs of natural products will also provide unique compounds of interest. The continued follow-up of empirical observations and changes in the structure of the already effective agents will also add to our array of new material for investigations.

Directed synthesis will also be aimed at improving those agents which have shown therapeutic effectiveness in the screen but have been abandoned later because of toxicological or other problems. The random but unique structures acquired from natural products--higher plants, fermentation products, algae, marine and insect products--will continue to be explored.

In addition to the search for "mitotically linked drugs", directed synthesis should also be aimed at affecting other phases of the cell cycle than the synthetic (S) phase or the mitosis (M) phase. The search for agents which have a high degree of organ localization should be undertaken and compounds containing this property should be considered as potential leads for chemical modification. Such specific localizing agents have been encountered with compounds containing the nitroso-moiety which may penetrate the blood brain barrier. A program has been developed to synthesize CNS active antitumor agents.

The Branch is keenly interested in trying to develop structure-activity correlations for those classes of compounds where sufficient examples which demonstrate some degree of activity are available. Recognizing that such studies are still in an early state of development, effort is continuing to investigate such studies using the techniques of regression analysis being developed by Dr. Corwin Hansch at Pomona College. Several problems do still exist in this area of investigation; such as, obtaining the physical-chemical measurements and the fact that the physical-chemical measurements are quite exact whereas the biological testing data has a considerable amount of variation. It must be recognized that this approach may not necessarily give immediate answers since it may be necessary to develop new parameters. However, it is hoped that such studies will focus attention on synthetic areas of greatest potential and provide the chemist with more directed synthetic approaches. This is not to be interpreted that new structural classes will be by-passed.

In addition to the above discussed effort in structure-activity correlation, the Branch is anticipating bringing together a group of top medicinal and organic chemists for an extensive review in specific areas similar to that done recently for folic acid antagonists and nucleosides. Conferences on the quinone moiety, alkaloids and alkylating agents were postponed due to budgetary limitations but are again being planned for the near future.

The Branch's laboratory operation has made significant technical progress on projects related to the synthesis of thalicarpine, azapurines and agents potentially active against brain tumors. Analytical research in high resolution NMR spectroscopy has also been investigated. A total of 95 compounds of known structure were submitted by the Drug Development laboratories for biological evaluation; forty-two of which were target compounds or their intermediates. In addition, studies on the decomposition of NSC-50741 [β-D-ribofuranoside, methyl 5-(2-chloroethylamino)-5-deoxy-2,3-O-isopropylidene-, hydrochloride] have been pursued.

The Branch manages a total of 40 contracts with industrial organizations, academic and research institutions, both domestic and foreign, totaling approximately six million dollars and covering the major areas as shown

under the primary responsibilities of the Branch. Of the 40 contracts, 36 are located in the continental United States and four are overseas contracts.

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees and academic institutions, research organizations and other governmental laboratories, since a substantial number of compounds are contributed to the Program on a voluntary basis from these sources. Contact is maintained with over 2000 suppliers, of which 1013 are industrial suppliers whose materials must be monitored on a "Commercial Discreet" basis.

The Drug Development Branch has maintained a steady flow of materials into the Program. These materials consist of chemicals, plant products, marine and insect products and fermentation products. Some of these materials have demonstrated confirmed activity in one or more of the primary screens (Step III) and have been assigned as candidates for preclinical toxicological evaluation (Decision Point 2). A number of Investigational New Drug Applications (IND) have been submitted to the Food and Drug Administration (See Table I).

In comparing 1970 with 1969, the data in Table I reflects a significant decrease in the number of crude plant extracts entering the Program. The number of crude extracts in the fermentation product category has remained about the same while there has been a slight decrease in the number of animal products. There has been a major decrease in the number of synthetic materials entering the program. Since the greatest number of active materials in the past has come from the synthetic category, it is expected that as the input of synthetics into the Program decreases, the total number of active materials will also decrease. This has been the case as we see an approximate twenty percent decrease in the number of synthetic Step III actives. The total number of confirmed actives, Step III, and the materials assigned for preclinical toxicological evaluation, reaching Decision Point 2, have shown significant decreases of 18% and 58% respectively. Even though the number of IND's filed with the Food and Drug Administration (see Table I) during 1970 remained the same as 1969, it is anticipated this number may drop in 1971 due to the decrease in the number of agents passing Decision Point 2 in 1970. The number of synthetic materials entering the screen in 1970 has markedly decreased. There were 115 synthetic confirmed actives, a decrease of 30. Three synthetic materials were selected for preclinical toxicological evaluation, Decision Point 2 (Table I), and 3 IND applications (Table I) were filed with FDA.

Of the 8,521 new and crystalline materials added in 1970 to the Program, 629 compounds (7.4%) were acquired under contract, 247 compounds (2.7%) were purchased, 3,037 compounds (35.7%) were voluntarily submitted from industrial sources and 4,608 compounds (54.2%) were from non-industrial sources. Of the total, 1,219 new materials were acquired by field collection trips.

Of the 3,987 plant extracts tested, approximately 79% were from contractors, and 119 crude extracts were active. Sixty-four new crystalline materials were isolated from plant extracts and 12 have shown confirmed activity (Step III). During this period one material of plant origin was selected for preclinical toxicological evaluation (Table I) and no IND's were filed (Table I).

From the 2,172 fermentation extracts, 104 demonstrated activity. Forty-eight were isolated in crystalline form and 7 have shown confirmed activity (Step III). Two fermentation products have been selected for preclinical toxicological evaluation (Decision Point 2) (Table I), and two IND's were filed (Table I).

Of the 587 crude animal products, 22 of these have shown activity. No crystalling materials have been isolated during this period, and none were approved for Decision Point 2 or filed for an IND.

The area of development and production of dosage forms for clinical use has continued to operate at a high level of effort. During the year 30 projects for formulation development were assigned to pharmaceutical contractors. Most of these projects have been completed and the resultant experimental dosage forms have been submitted for further preclinical testing.

Nineteen new dosage forms were developed, produced, and cleared for clinical distribution in 1970. Of these 13 were formulated for parenteral use and 6 were processed for oral administration. The production record achieved by the formulation contractors accounts for 1,085,160 compressed tablets, 206,395 hard gelatin capsules and 197,185 vials. A total of 466,717 drug units (vials and bottles) were received in 1970. During the same period 430,380 drug units were distributed to clinical investigators throughout the world. An average inventory of 99 different compounds, 186 different formulations involving 294 dose forms have been maintained.

In certain situations it is desirable to evaluate more than one drug or a drug and placebo under the same conditions. This is done by "double-blind" clinical studies which require that the materials used are to be formulated in dosage forms that cannot be differentiated from each other except by code. There are 21 coded studies on inventory.

The Branch, upon request, has reviewed and commented upon 152 Grant Proposals. The opinions expressed have been used extensively by the Grant Study Sections on their evaluation of the Proposals. Such comments may include the number of materials submitted for testing, indicating those compounds showing promise as potential antitumor agents.

Also upon request, the Branch has reviewed 6 patent applications. The applications are reviewed as to novelty, expected utility and need to protect the public's interest by patent. The opinions and recommendations are used by the NIH Patent Section in their consideration of the application.

Future Objectives

A great deal of attention is being directed toward analysis of the Branch's activities in respect to program objectives. The entire program staff is responding to the "linear array" (the logical steps in developing new drugs). This is resulting in a more efficient and accelerated drug program. Such a process focuses attention upon the most direct route to preparing a drug for clinical trial. With more sharply defined objectives the staff is reviewing the contractural efforts with regard to present and anticipated program needs.

We expect to bring into being the full support of the Branch's newly developed laboratories and staff on program oriented objectives in synthesis, isolation, instrumental and analytical analysis, and formulation research.

Efforts will be continued, on an increased basis, to design and develop new structural classes of compounds directed toward eradicating both rapid and slow growing tumors. In the search for novel structures, unique natural products will also be searched. We are broadening our worldwide surveillance for information and for new drugs arising from the scientific community, which will also include biologically active materials not yet evaluated in the cancer program.

The basic extraction protocol of natural products is under study, especially plant materials, in the hope of enriching the active component in the crude extract prior to initial testing, since the present system will only detect materials which are active at a very low dose level. A greater number of active materials should be encountered which would otherwise be missed.

As the program needs increase for large quantities of natural products, efforts will continue to be made to prepare by synthetic methods natural products which are either difficult or impossible to secure from natural sources. Such methods will also reduce the cost and time for procurement as well as provide interesting chemical modifications.

In order to further some of the current areas of interest, effort will be continued in developing structure-activity correlations. Such correlations will be accomplished via comprehensive reviews on specific chemical classes by a group of chemical advisors and other experts in the field as well as via the newly developed techniques of computerized regression analysis. These studies will bring to light not only analogs omitted from the correlation but also new analogs that are needed for evaluation. With the study to computerize chemical data retrieval well underway, it is anticipated that the implementation of such a system will be undertaken in the near future. The computerization of chemical data retrieval will facilitate the accessioning of compounds entering the program as well as other operations of the Branch.

As the pharmacological and clinical studies expand, there will be a corresponding increase in the need for quality control on bulk chemicals, experimental dose forms and final clinical dose formulations. In order to retain the proper control on these materials a continued refinement of the methods of analysis will be made.

The Branch recognizes the value of the scientific community and their many contributions to program needs and we plan on continuing the practice of scientific seminars and "workshops". The objectives of these workshops would be to give guidance to the rational selection of scientific problems, the approach to their solution, and the evaluation of the results. The participants of such meetings would be outside consultants, principal investigators of contracts and the staff.

Of special interest to the Branch is the establishment of an annual contractors' meeting in the area of synthesis, natural products, quality control and drug formulation. The objective would be to pool the contractors' talents in solving program problems and the exchange of technical information in the respective areas.

Summary of Branch Achievements

In summary, the following were achieved during the calendar year 1970 by the Drug Development Branch:

1. A total of 15267 new substances (Table I) was obtained for screening or other evaluation;

   a. 8409 synthetics

   b. 2172 antibiotic cultures

   c. 3987 plant materials

   d. 587 animal materials

   e. 112 other crystalline materials

2. A total of 756 compounds were prepared under contract;

   a. 127 were resynthesized by the preparation laboratory contracts

   b. 576 were synthesized as new compounds from synthesis and prep lab contracts

   c. 53 were from the natural product laboratory contracts

3. Seven new substances were assigned to Decision Point 2 (Table I and II). (Candidates for preclinical toxicology);

   a. 3 fermentation products

   b. 1 plant product

   c. 3 synthetic compounds

4. Five drugs were assigned IND numbers (Table I and III).

   a. 2 fermentation products

   b. 3 synthetic compounds

5. One hundred thirty-five compounds were assigned to Step III (Table I).

6. Analytical-quality control determinations were made on 143 different lots of 86 different compounds and 81 lots of clinical formulations.

7. Twelve radioactive labeled compounds (Table V) were procured.

8. Thirty dose formulations were developed of which 19 entered clinical trial.

9. Approximately 1,488,740 formulated items were prepared;

   a. 1,085,160 compressed tablets

   b. 206,395 hard gelatin capsules

   c. 197,185 vials

10. Forty contracts (some multi-phase contracts) were supervised by the Branch;

   a. 8 preparation laboratories

   b. 9 new synthetics

   c. 10 plant products

   d. 3 fermentation products

   e. 2 analytical-quality control

   f. 4 drug formulations

   g. 4 miscellaneous

11. There were 152 Grant Proposals and 6 Patent Applications reviewed and commented upon.

12. Samples were received from 47 new industrial concerns.

13. Materials were received from 142 new non-industrial sources.

14. Sixty-five manuscripts by contractors were cleared for publication.

15. Six manuscripts were published by the Branch staff.

16. Seven seminars were sponsored.

TABLE I

MATERIALS ACCESSIONED AND ACTIVITY REPORT [1]

	CRUDE EXTRACTS NO. SUBMITTED		CRUDE EXTRACTS NO. ACTIVE		CRYSTALLINE MATERIAL SUBMITTED		STEP III[2]		DECISION POINT 2[3]		IND FILED	
	1969	1970	1969	1970	1969	1970	1969	1970	1969	1970	1969	1970
Fermentation Products	2184	2172	148	104	36	48	8	7	3	3	—	2
Animal Products	757	587	17	22	5	—	—	1	—	—	—	—
Plant Products	5109	3987	83[4]	119[4]	41	64	13	12	3	1	2	—
Synthetics	—	—	—	—	9738	8409	145	115	8	3	3	3
Total	8050	6746	248	245	9820	8521	166	135	14	7	5	5

[1] Based on calendar year 1970.

[2] Showing confirmed activity.

[3] Selected as candidates for preclinical toxicological evaluation and clinical trial. (formerly Step IV-A).

[4] Individual species.

TABLE II

COMPOUNDS PASSING DECISION POINT 2 DURING CALENDAR YEAR 1970

NSC NO.	NAME	SOURCE
77037	d-Tetrandrine	Plant Product
106977	L-Asparaginase, from *Erwinia caratorvora*	Fermentation Product
112907	Tetrahydrouridine	Synthetic
118994	Diglycolaldehyde, α-(hydroxymethyl)-α'-(6-hydroxy-9H-purin-9-yl)-	Synthetic
129185	4-Hexenoic acid, 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl- (Mycophenolic acid)	Fermentation Product
135758	2,5-Piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride (Antibiotic 593-A)	Fermentation Product
136592	Dichloroallyl lawsone, sodium salt	Synthetic

TABLE III

NEW IND APPLICATIONS FILED IN 1970

NSC NO.	NAME	IND NO.
1895	Guanazole	7065
71261	Beta-TGDR	6874
107392	Picolinaldehyde, 5-hydroxy-, thiosemicarbazone	6557
123127	Adriamycin	7038
125066	Bleomycin	6649

Serial No. NCI 3580
1. Chemotherapy, CCNSC
2. Drug Development Branch
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemical Research in the Development of New Anticancer Drugs

Previous Serial Number: NCI 3580

Principal Investigator: John S. Driscoll

Other Investigators: Kenneth Paull, Tadashi Hirata, Ashwani Malhotra, Victor Marquez, Robert Engle, Harry B. Wood, Jr., Jonathan Hartwell, Alfred Stanley and Abraham Goldin

Man Years

Total: 4.8
Professional: 4.2

Project Description:

General Objective:

The objective of this project is the discovery of new types of clinically useful anticancer drugs. This goal is pursued using leads developed through structure-activity correlations, the data from the random screening of compounds, and literature biomechanistic rationale.

Candidate drugs can originate either as synthetic materials or may be isolated from active natural product extracts. Natural products with proven effectiveness are synthesized.

Specific Objectives:

1. The synthesis of thalicarpine (NSC-68075).

2. The synthesis of bridge-head nitrogen heterocycles.

   a. The synthesis of 5-azapurines, unique triazoles, and the correlation of their structures with NMR spectra.

3. The preparation and structural determination of the L-1210 active component in decomposed NSC-50741.

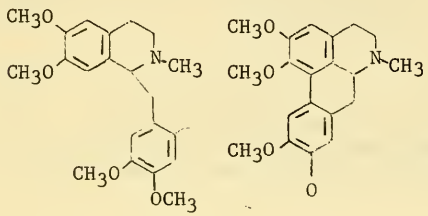
4. The synthesis of drugs designed for use against brain tumors.

5. The synthesis of selected folic acid derivatives.

Major Findings:

1. The Synthesis of Thalicarpine (K. Paull and R. Engle)

Thalicarpine (NSC-68075) is a plant alkaloid which is almost ready for Phase I clinical trial. Because of the difficulties in procuring the material from natural sources, a total synthesis of this agent (1) was undertaken.



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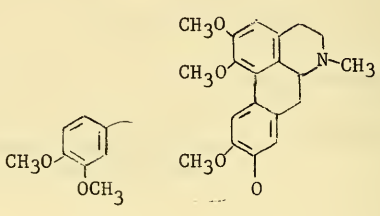


(2)

Ten of the fourteen steps in the synthetic scheme have been successfully accomplished. Highlights were the preparation of a key diphenyl ether intermediate in >45% yield, and the closing of the aporphine ring to provide intermediate (2).

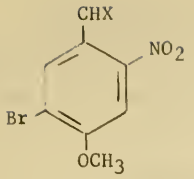
An unusual nucleophilic displacement reaction was also observed and characterized. When the potassium salt of 3,4-dimethoxyphenol was reacted with (3,x=H_2), the bromine was displaced, as expected. When (3,x=0) was reacted, however, the nitro group was displaced in preference to the bromine. When the dimethylacetal of the aldehyde was prepared {3,x=$(OCH_3)_2$}, and reacted, displacement of the bromine was again the preferred reaction path.

Seventeen intermediate reaction products prepared on this project were submitted for biological testing.

2. The Synthesis of 5-Azapurines and 1,2,4-Triazoles (T. Hirata, H. B. Wood, Jr., and A. Goldin)

A number of candidate antitumor drugs possess bridge-head nitrogen atoms in their structures. The 5-azapurines, members of this family, were chosen for investigation for a number of reasons, among which was that the sym-triazoles, which are intermediates, might themselves be good antitumor drug candidates.

Attempts to prepare the 6-mercapto- or 6-chloro-5-azapurine derivatives from 5-azahypoxanthine by conventional means failed. The 5-azapurines (4) and (5) were finally prepared from appropriately substituted 1,2,4-triazoles.



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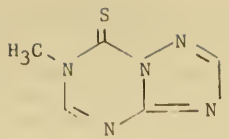

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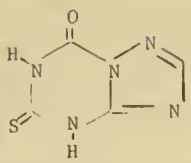


(5)

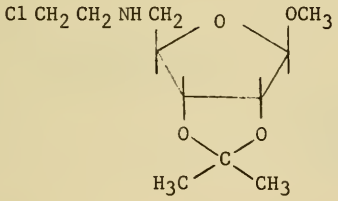
During the course of this work, many unique derivatives of 3-amino-1,2,4-triazole were prepared and characterized. The difficulties described in the literature concerning the assignment of structure among the many possible N-substituted isomers was resolved by the use of high resolution NMR spectroscopy.

A useful rearrangement reaction was discovered and exploited to yield previously unobtainable triazoles.

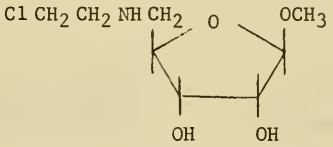
Eighteen triazoles and two 5-azapurines have been submitted for evaluation in the L-1210 screen.

3. The Preparation and Structural Determination of the L-1210 Active Component in Decomposed NSC-50741 (A. Malhotra)

Pure NSC-50741 has no L-1210 activity. A five-year old, decomposed sample, however, was found to have good, reproducible activity. Since all of the decomposed material had been consumed, the objective of this project was the reproduction of the active material, and the determination of the structure of the active component.



(NSC 50741)



(6)

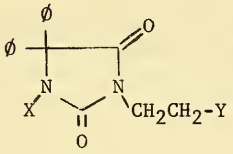
Aqueous solutions of NSC-50741 were carefully studied at several temperatures (40°, 60° and 85°). Samples were taken at regular time intervals and were evaluated in the L-1210 test system. Optimum reaction conditions (60°, 5 hours) were determined. These products gave test results of 150-185% T/C. The unfractionated lyophylized product has given values as high as 170 and 187%. While fractionation of the reaction product into three components by Dry Column chromatography was successful, the pure active component has not yet been identified. Compound (6) has been identified as one of the components by mass spectral, NMR and elemental analysis. This compound is described in the

literature as too unstable to isolate. A second component is starting material plus a very toxic material with approximately the same R_f value. The third component is aromatic in character and is suspected to be a pyridine derivative. The third component has given an L-1210 T/C value as high as 205%. A total of 92 reaction mixtures and fractions have been submitted for L-1210 testing, while 45 were evaluated in the KB test system.

4. The Synthesis of Drugs Designed for Use Against Brain Tumors

a. Drug Design and Synthesis (V. E. Marquez and J. L. Hartwell)

Based on a thorough literature search of all central nervous system (CNS) active drugs, an experimental program was designed incorporating (a) the important properties of drugs that enter the brain [ability to cross the blood-brain barrier (BBB), high brain to plasma concentration ratio] with (b) the features of a potential antitumor agent [high tumor to brain concentration ratio, alkylating properties, enzymatic inhibition]. From nineteen promising families of compounds, which are known to cross the BBB, three were judged to have the optimum chances for successful modification. Compounds were designed and synthesized in the diphenylhydantoin (7) phenothiazine (8) and dibenzazepine (9) series.



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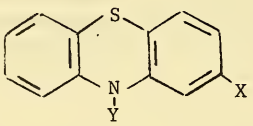

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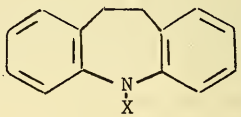


(9)

a. X=H, Y=OH
b. X=H, Y=OSO_2CH_3
c. X=H, Y=Cl
d. X=NO, Y=Cl

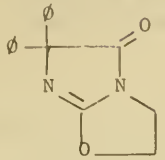
a. X=Cl, Y=$(CH_2)_2CH_2Cl$
b. X=Cl or CF_3
Y=$(CH_2)_2CH_2N(CH_2CHCH_2)_2$ (O)

X=$(CH_2)_2CH_2Cl$

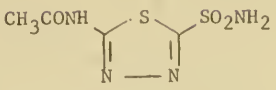
Compounds 7a-c, 8a and 9 have been prepared and submitted for L-1210 testing. The cyclic nitrosourea (7d) has been prepared and characterized on a small scale. Compounds (8a) and (9) are intermediates to compounds with side chains characteristic of Epodyl (8b), an agent which has been used in brain tumor therapy.

An unusual hydantoin (10), which gives a very strong alkylating agent test, was discovered, characterized, and submitted for testing. Preliminary studies in the acetazolamide series (11) have also begun.

Seven target compounds have been submitted for screening on this project.



(10)



(11)

b. Screening of Psycotropic Drugs as Potential CNS Antitumor Agents (F. Quinn and J. P. Davignon)

A program has been designed and initiated to obtain and screen the 690 CNS active drugs cataloged in "Psycotropic Drugs and Related Compounds", E. Usdin and D. H. Efron, NIMH, PHS, DHEW, PHS Publication 1589, 1967. It is hoped that any intraperitoneally L-1210 active compounds discovered will have a high probability of also being intracerebrally active.

5. The Synthesis of Folic Acid Derivatives (K. Paull and R. Engle)

Two derivatives of homofolic acid, incorporating changes in the aminoacid portion of the molecule, were synthesized and submitted for testing.

Serial No. NCI 3581
1. Chemotherapy, CCNSC
2. Drug Development Branch
3. Office of the Chief
4. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Analytical Chemistry of New Anticancer Drugs

Previous Serial Number: NCI 3581

Principal Investigator: John S. Driscoll

Other Investigators: Li-Ming Twanmoh, Robert R. Engle, Clifford A. Hewitt, and Harry B. Wood, Jr.

Man Years

Total: 1.2
Professional: 1.2

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;

2. establish the identity and purity of bulk and formulated drugs;

3. determine important physical and chemical properties of new anticancer drugs;

4. study reaction mechanisms and structure-activity relationships.

Methods Employed:

Emphasis is placed on modern instrumental techniques. Primary emphasis is placed on the use of high resolution NMR spectroscopy as an analytical tool. Chromatographic techniques, as well as infrared and ultraviolet spectroscopy, are also extensively employed.

Major Findings:

1. Structural Studies (L-M. Twanmoh and H. B. Wood, Jr.)

a. Triazoles and Azapurines

Approximately 65 samples in this series were studied by NMR spectroscopy. Protons attached to position-2 in sym-1,2,4-triazoles were discovered to be highly deshielded, with chemical shifts of δ=14ppm. This finding has been very useful in the determination of the structures of isomeric derivatives which previously had been almost indistinguishable.

b. Potential CNS Antitumor Agents

The structure and purity of 30 samples in the diphenylhydantoin, phenothiazine and dibenzazepine series were determined by NMR spectroscopy. The most important signals in these compounds were found to be the characteristic side chain methylene protons.

c. Aminosugars

NSC-50741 is a ribosyl mustard derivative. While the pure compound is inactive, thermal degradation in aqueous solution gives a complex, but L-1210 active mixture. After various components were isolated, NMR, IR and UV spectroscopy are used to identify these materials. One of the pure materials isolated from the reaction mixture was shown by NMR spectroscopy to be a compound described in the literature as being too unstable to be isolated. Approximately 35 samples were studied.

d. Isoquinolines and Folic Acid Derivatives

Forty-five samples, mostly intermediates in the synthesis of thalicarpine (NSC-68075), were characterized by NMR spectroscopy. The successful closure of the aporphine ring during this complex, multistep synthetic project is monitored by the appearance of a unique aromatic proton signal at δ=8.16ppm.

e. Miscellaneous Structural Studies

(1) Indian Compounds

Four nitrogen heterocycic compounds were subjected to high resolution NMR analysis for Dr. N. Anand, Central Drug Research Institute, Lucknow.

(2) Amino Acids and Peptides

Ten samples of low molecular weight peptides were characterized for a CCNSC contractor by NMR spectroscopy.

(3) National Heart and Lung Institute Compounds

The spectra of four unknown compounds, available in very small amounts, were determined for Dr. Sokoloski by the use of Fourier transform NMR spectroscopy.

This technique, made possible by a unique accessory to our 100 MHz spectrometer, allows rapid signal to noise discrimination.

2. Reaction Mechanisms (L-M. Twanmoh)

a. Enzyme-Drug Interactions

An enzyme-binding study was carried out in cooperation with Dr. F. Friedberg of Howard University. His objective was to learn NMR techniques as related to metal-enzyme binding, while our objective was to learn enzymatic techniques which might be applied to antitumor drug-enzyme interactions. While the calcium-(α-chymotrypsin) system chosen for study was found not to be optimum, both objectives were partially accomplished with a minimum expenditure of effort. Previously unrecognized practical problems involving the use of D_2O as a solvent for Fourier transform studies were encountered and resolved.

b. Non-enzymatic Transamination Reactions

The enzymatic transamination of H^3 labeled glutamic acid results in non-radioactive α-ketoglutaric acid. A model study was carried out with Dr. D. Cooney (C,LT) to discover if NMR spectroscopy might be useful in determining whether unique intermediates play a role in the mechanism of transamination. The aluminum catalyzed reaction between aspartic acid and pyridoxyl in D_2O was studied at 50^0. The reaction was carried out in a NMR tube, and was followed by Fourier transform NMR spectroscopy because of the low solubilities of the reactants. It was concluded that the loss of β-protons in the transamination reaction was most likely the result of a fast keto-enol tautomerism in the reaction product.

c. Structure-Activity Studies of Methotrexate Derivatives

A study was recently initiated to determine the feasibility of correlating biological activity with NMR data from compounds with substituents in the benzene ring. It is hoped that information can be obtained concerning the degree of conjugation between the N-10 nitrogen and the carbonyl group para to the nitrogen function.

3. Assay and Physical Property Studies (L-M. Twanmoh, C. A. Hewitt, and V. E. Marquez)

a. 3-α-Hydroxy-5-β-androstan-17-one (Etiocholanolone)

The Clinical Center Pharmaceutical Development Service had drug samples with different activities from two sources. These lots were found to have identical NMR spectra which were consistent with the structure of the compound.

b. Daunomycin

Assay and purity studies were conducted using extraction, IR and UV

techniques. The lot assayed was found to be acceptable.

c. Streptovitacin A (NSC-39147)

An IR spectral analysis of two lots of this cycloheximide antibiotic showed the samples to be comparable. The spectrum was consistant with the structure.

d. Miscellaneous Studies

The solubility properties of two Australian compounds (NSC-113089, 114347), which had been reported to be very unusual, were studied and determined to be normal.

The variation in activity of NSC-68105 was traced to the submission of different derivatives under the same NSC number.

CHEMICAL AND DRUG PROCUREMENT SECTION

DRUG DEVELOPMENT BRANCH

Scope

The function of the Chemical and Drug Procurement Section is to acquire synthetic compounds for antitumor evaluation, to procure large quantities for advanced screening, confirmational testing, toxicological and pharmacological evaluation, and clinical trial in humans, and to procure radiolabeled materials needed for pharmacological and clinical studies.

The work of the Section is divided into the following three principal areas:

1. The Synthesis Activities, which provide new compounds for primary screening;

2. The Preparative Activities, which synthesize

   a. bulk chemicals and drugs

   b. radiolabeled materials

   for toxicological, pharmacological and clinical investigations, for confirmational testing and for completion of primary evaluation; and

3. Analytical-Quality Control Activities, which provide the analytical check on the identity, assay and purity of compounds and formulated drugs entering the pharmacological and clinical programs. Solubility and stability data are also acquired as is structural analysis.

Staff

The staff of the Chemical and Drug Procurement Section is presently composed of three professional people and one secretary. One professional person is involved full time with the quality control activities of the Section. The professional staff members serve as project officers on the various contract activities of the Section as well as on other contracts that are monitored by the Drug Development Branch.

Synthesis Activities

The objective of the synthesis laboratories is to provide the program with new chemical agents for anticancer evaluations. The laboratories are designed to supply compounds of diverse structures and complexity. The compounds selected for preparation are based upon the following criteria:

1. Modification of clinically active compounds;

2. Leads generating from the screening program;

3. Biological and biochemical rationale;

4. Leads from the literature;

5. Compounds to explore structure-activity relationships;

6. New structural types; and

7. Agents to resolve questions on chemical and biochemical mechanism and mode of action.

The efforts of the synthetic program are channeled into many areas of investigation. The following are some examples:

(1) A significant amount of the synthetic effort of the Section has been directed toward the synthesis of natural products, their analogs and derivatives. Several products are not available in the quantities necessary for complete evaluation from natural sources, and if the structure is known, attempts are made to prepare the material by synthetic means where feasible. Many times the investigation of a synthetic route to the preparation of a natural product affords, or affords the means to, the preparation of analogs which may be more active and interesting than the natural material. It may also be desirable to prepare synthetic analogs and derivatives of naturally occurring material to develop the parameters of an interesting lead or in the hopes of reducing adverse toxicity or metabolism while retaining activity. Recognizing that natural materials are one source of interesting structures and compounds, quite often analogs and derivatives of these can be procured only by synthetic means.

Camptothecin, as well as several other compounds which have demonstrated in vivo activity, contains an α-hydroxy-lactone moiety. There is some indication that this structural moiety is important for activity. The Section has therefore continued the preparation of compounds containing an α-hydroxy-lactone moiety. The synthesis of some compounds of this type was achieved during this report period.

The total synthesis of the naturally occurring alkaloid, camptothecin, continued to be of interest during the report period. The soluble sodium derivative of this material, prepared from camptothecin, is undergoing clinical evaluation. Since the quantity of camptothecin which can be obtained from natural sources is very limited and its extraction from plant material is very expensive, the total synthesis of this alkaloid is of interest. Three contracts, Georgia Institute of Technology, University of Michigan and Midwest Research Institute are devoting efforts towards developing a practical synthesis for the preparation of this material, its analogs and derivatives. Unfortunately, the method for the synthesis of camptothecin has not been achieved to date but work is progressing. Upon completion of the synthesis, some analogs and derivatives will also be prepared.

Several of the clinically active compounds, such as Daunomycin, Mitomycin C, Streptonigrin and Actinomycin D, and materials which have demonstrated interesting antitumor activity in the screen such as Lapachol, have a

quinone moiety present. The Section has therefore undertaken work in the synthesis of quinone-type molecules. This work was done at Midwest Research Institute and Stanford Research Institute. The synthesis of some compounds of this type was achieved during this report period.

In addition to the above-mentioned work based on the quinone moiety of Daunomycin, other synthetic analogs and derivatives of this clinically active antibiotic have been prepared by Stanford Research Institute. Analogs of both the aglycone, daunomycinone, and the sugar, daunosamine, have been prepared. Also, a series of amino and carbonyl derivatives of daunomycin have been prepared. Work on the preparation of analogs and derivatives of daunomycin and adriamycin is continuing.

Work has continued on the preparation of analogs and derivatives of the active alkaloid ellipticine. The main effort during this report period has been devoted to the preparation of close relatives bearing various combinations of substituents on the 1, 5, and 6 positions. Work in the ellipticine area was done at Stanford Research Institute and Central Drug Research Institute (India).

During this report period, some of the synthetic effort of Microbiological Associates, Inc. has been directed toward the total synthesis of the in vivo active alkaloid, thalicarpine. The necessary preclinical work on this alkaloid has recently been completed and its clinical evaluation is scheduled to start in the near future. Since the quantity of this compound that can be obtained from natural sources is rather limited and its extraction from the plant is very expensive, the total synthesis of this alkaloid is of interest and continues to be pursued.

Some of the synthetic effort of the Section has been devoted to the preparation of some structural moieties of the antitumor antibiotics, Bleomycin. Bleomycin is a mixture of closely related antibiotics, the chemical structures of which have not been completely elucidated. It is known that bleomycins contain polypeptides composed of a substituted bithiazole among others. Several bithiazoles and related thiazoles have been prepared by Midwest Research Institute.

(2) A second major effort of the synthesis program has been the preparation of analogs and derivatives of folic acid. Resistance to folic acid antagonists in L-1210 leukemia is frequently associated with a marked increase in the enzyme dihydrofolate reductase. Since this change is limited to tumor cells, the provision of a fraudulent substrate to the tumor cell would lead to the production of a fraudulent coenzyme, thus producing a lethal synthesis in the tumor cell. Homofolic acid was prepared as a potential fraudulent substrate for dihydrofolate reductase in resistant tumor cells. Tetrahydrohomofolic acid, the end product of the reduction of homofolic acid by dihydrofolate reductase, has also been prepared and is a striking inhibitor of thymidylate synthetase. Tetrahydrohomofolic acid is presently being obtained in large quantities for further evaluation.

New folic acid analogs and derivatives have been prepared by Midwest, Southern, and Stanford Research Institutes and our intramural laboratory program. The structure of the folic acid molecule has been divided into five parts, (a) Ring A (pyrimidine) of the pteridine ring, (b) Ring B of the pteridine ring, (c) the Bridge, (d) the Benzene portion (Ring C), and (e) the Amino Acid moiety. Modifications have been undertaken in each area. Some examples of folic acid analogs prepared during this report period are (a) 1- and 3-deazamethotrexate (b) the pteridine ring in methotrexate replaced by 2,4-diaminopyrimidine (c) 10-deazafolic acid (d) N^{10}-allyl analog of methotrexate, (e) the p-aminobenzoic acid moiety in folic acid replaced by p-aminophenyl acetic acid (f) 2',5'-dichloro-methotrexate (g) 3'-trifluoromethylmethotrexate (h) 3',5'-dimethyl-methotrexate and (i) meta analog of methotrexate. In addition, the pteroic acid intermediates for the preparation of 7-azafolic acid and homomethotrexate have been obtained.

(3) Some of the synthetic effort is devoted to the synthesis of nucleosides. The area of nucleosides has already provided a number of very interesting compounds capable of suppressing tumor growth in experimental animals and producing favorable clinical response in certain types of human cancer. Cytosine arabinoside and 5-FUDR are nucleosides which have demonstrated clinical activity. However, further chemical studies are needed to provide nucleosides of greater specificity and less toxicity and which are resistant to enzymatic degradation in the cancer patient.

In addition, the study of nucleoside metabolism may be expected to contribute to the design of chemotherapeutic agents with antiviral activity against tumor-inducing viruses and may help elucidate many cellular processes in the synthesis of DNA and therefore in cell division. Nucleoside antimetabolites may function by inhibiting the incorporation of normal constituents in RNA or DNA and preventing the formation of enzymes necessary for biosynthesis of the tumor nucleic acids. Also, the conversion of nucleosides to nucleotides may be structurally less restrictive than the conversion of the free base to the corresponding nucleotides.

A wide variety of structural modifications and derivatives of nucleosides has been prepared by Southern Research Institute, Stanford Research Institute and the University of Utah and submitted for evaluation during this report period. These include (a) analogs of the nucleoside antibiotics such as tubercidin, toyocamycin, sangivamycin, formycin, formycin B, and puromycin, (b) pyrrolo[2,3-d]pyrimidine nucleosides (c) tricyclic nucleosides (d) nucleosides of pyrazolo[3,4-d]pyrimidine, pyrazolo[4,3-d] pyrimidine, imidazo[4,5-c]pyridine and imidazo[4,5-b]pyrazine (e) indazole, imidazole and benzimidazole nucleosides (f) nucleosides containing thio sugars, amino sugars, and unsaturated sugars (g) uronic acid nucleosides (h) "carbocyclic sugar" nucleosides derivatives (i) "carbon-carbon nucleosides" and (j) nucleosides with natural bases and fraudulent sugars, with natural sugars and fraudulent bases, and with fraudulent bases and sugars.

(4) Some of the effort of the Section has been directed towards the synthesis of other potential antimetabolites. One such structural type is the

imidazole. Two imidazole-containing compounds have demonstrated activity against L-1210 leukemia and are undergoing clinical evaluation. In addition, some compounds of this type have been shown to have broad spectrum antifungal and antibacterial activity. Thus, additional imidazole analogs and derivatives have been prepared and are being synthesized for evaluation as antitumor agents.

(5) The class of compounds known as N-nitrosoureas are active against leukemia and some possess the ability to cross the blood-brain barrier. Of special interest are the results from intracerebrally-inoculated L-1210 leukemia, Lewis Lung carcinoma, and the use of maximal single dose (one day only) treatment schedules. The testing in rodents with some compounds of this type has resulted in a significant number of cures based on thirty-day survivals. Additional examples of this structural type have been synthesized by Southern Research Institute and submitted for antitumor evaluation.

(6) A limited amount of effort has been devoted to the synthesis of analogs of guanazole, which has demonstrated activity against L-1210 leukemia and is currently undergoing clinical evaluation.

(7) Structural modifications of the in vivo antileukemic active nicotinohydroxamic acid has been undertaken. Interest in the hydroxamic acid type of compound has been enhanced by the recent observation that some compounds of this type are also inhibitors of ribonucleoside diphosphate reductase. Several compounds of this class have been prepared and work in this area is continuing.

(8) A limited amount of effort has been devoted to the isolation and synthesis of metabolites of cyclophosphamide. This clinically effective agent is itself inactive and becomes effective only after appropriate activation in vivo. During this report period the metabolite, 4-ketocyclophosphamide, was isolated and synthesized by Southern Research Institute. Work in this area is continuing in an effort to determine the mechanism of activation of this active agent and to isolate and synthesize the active metabolite(s).

One very important advantage of synthesis contracts of this type is that they are placed with laboratories which are able to offer a quick response to a new lead and move quickly into an area of research generated by the Chemotherapy Program and the various clinical groups. The broad experience and mature judgment in synthetic chemistry of these laboratories place them in a unique position for all types of synthesis. These contractors have provided a great number of compounds with a wide variation in chemical structure.

During the past year, six new compounds passed Decision Point 2, of which three were synthetic materials. These new synthetic compounds are listed in Table II.

The Chemical and Drug Procurement Section directs nine contracts for the chemical synthesis of new compounds for the Program (Table IV).

TABLE IV

CONTRACT LABORATORIES FOR SYNTHESIS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Central Drug Research Institute	Dhar	01-004-1 (PL 480)
Georgia Institute of Technology	Zalkow	PH43-68-1288
University of Michigan	Burckhalter	NIH-69-2004
Microbiological Associates, Inc.	Northrop	PH43-68-1283
Midwest Research Institute	Cheng	PH43-65-94
Southern Research Institute	Montgomery	NIH-71-2021
Stanford Research Institute	Goodman/Henry	NIH-71-2070
University of Utah	Townsend	PH43-65-1041

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories, and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not available from the original supplier or on the open market. These laboratories differ from the synthesis contract laboratories in that the synthesis contracts are devoted entirely to the preparation of new compounds with diverse chemical structures for primary screening. The preparative laboratories also perform the large scale synthesis, extraction, and isolation of materials from natural sources. The laboratories were selected on the basis of ability of the principal investigator, the facilities available, the capability of the company in the field of chemistry related to the Chemotherapy Program and the adaptability to synthesis of all types of compounds.

The preparation laboratories are also used to obtain data for the preparation of the necessary quantities of clinically-important chemicals and to develop the most economical means for their preparation. Usually methods and/or references for the synthesis of the compounds requested are supplied to the contractors by the Section. It should be pointed out that many methods of synthesis which are practical for a small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies have also been performed by the preparation laboratories upon request. In addition, some of these contractors have provided material in formulated dose form.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs which may be needed for the Program.

(1) Bulk chemical and drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for resynthesis for five major reasons:

(a) For clinical investigations;

(b) For preclinical toxicological and pharmacological evaluation;

(c) For maintaining a stock of chemicals of clinical and preclinical interest for study in the Program;

(d) For completion of screening or confirmational testing; and

(e) For use as intermediates in further synthesis.

The compounds so chosen are placed on priority lists based upon their relative importance, with the toxicological and clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from 1 gram to occasionally as much as 75 kilograms. Factors governing the amounts depend upon such considerations as: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the clinician in the quality of the drug he uses.

The Chemical and Drug Procurement Section, through the preparative laboratories, directed the resynthesis of 128 compounds totaling 127 kilograms during the calendar year 1970. Included in this group were 18 compounds scheduled for or undergoing clinical evaluation totaling 121 kilograms. In addition to the compounds resynthesized, 97 new compounds were also prepared by the preparative laboratories. During this report period the Section also purchased 12 compounds scheduled for or undergoing clinical evaluation totaling 37.7 kilograms.

(2) Radiolabeled Materials

The continued importance being placed on pharmacological and drug distribution studies has maintained the need for radiolabeled chemicals and drugs. These agents are valuable tools in such studies and the Section coordinates their procurement and distribution.

One contract (PH43-65-1044) is devoted to the procurement of radiolabeled materials for use in preclinical pharmacological and clinical studies. If available, the required labeled materials are purchased from commercial sources. Materials not available from commercial sources are prepared by this preparative laboratory. This contract also handles the storage and distribution and performs the necessary analytical work for all labeled

materials, whether prepared under the contract or acquired from other sources. All requests for radio-active materials are reviewed by a committee in relation to proposed use prior to assignment to the contract for procurement. During this report period, the Section procured from all sources a total of 12 radiolabeled materials (Table V) and directed their distribution. Forty-three shipments of radio-active substances were made during calendar year 1970.

TABLE V

RADIOLABELED MATERIALS PROCURED

NSC NO.	RADIOLABELED MATERIAL	LABEL
740	Methotrexate	3',5'-H^3
755	9H-Purine, 6-thiol-	S^{35}
26271	Cyclophosphamide	C^{14} (side chain)
52695	Phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-(3-hydroxypropyl)- compound with cyclohexylamine (1:1)	C^{14} (ethylene label)
52695	Phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-(3-hydroxypropyl)- compound with cyclohexylamine (1:1)	C^{14} (propylene label)
63878	Cytosine, 1-β-D-arabinofuranosyl-,	H^3
63878	Cytosine, 1-β-D-arabinofuranosyl-,	2-C^{14}
71795	Ellipticine	1-C^{14}
85998	Streptozotocin	H^3 (Ring label)
95441	Urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso	C^{14} (Ethylene label)
109724	1,3,2-Oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)-amino]tetrahydro-, 2-oxide	C^{14}
117613	Norvaline, 5-diazo-4-oxo-, L-	C^{14}

During this report period the Section directed eight preparation laboratories (Table VI).

TABLE VI

CONTRACT PREPARATIONS LABORATORIES

FOR CLINICAL AND EXPERIMENTAL COMPOUNDS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Aerojet-General Corp.	Jagow	PH43-68-1300
Collaborative Research, Inc.	Friedman	PH43-66-506
Merck and Co.	Babson	PH43-62-479
Monsanto Research Corporation	Yanko	PH43-65-1044
Monsanto Research Corporation	Ellard	PH43-67-658
Stanford Research Institute	Goodman/Henry	NIH-69-37
Starks Associates, Inc.	Starks	PH43-62-484
Starks Associates, Inc.	Starks	PH43-68-1275

Quality Control

The Branch is keenly aware of the need for providing bulk chemicals and formulated drugs of highest quality for use in clinical investigations and preclinical evaluations. The purpose of quality control, based on rigorous chemical analytical methods, is to evaluate these materials as to their identity, assay and purity. All lots of bulk materials to be used in preclinical toxicological and pharmacological or in clinical studies are sent for analysis before the material is released for use or formulation. Also, quality control data is obtained for all formulated material prior to its release to the clinical investigators.

It should be pointed out that the Chemotherapy Program deals with a great diversity of chemical compounds, many of which are entirely new with no methods of assay available. Very few compounds are assayed on a routine basis; thus, in many cases, methods of assay must be developed. These assay methods are made available and serve as an invaluable tool to the investigator using the particular compound. Also, the program on analytical studies of anticancer compounds became involved in working out analytical methods for many of the compounds in biological media such as blood and urine.

Among the techniques that are most commonly used in the drug analysis work are: spectroscopic determinations (ultraviolet, visible, infrared and nuclear magnetic resonance), chromatographic determinations (paper, thin layer and gas-liquid) and titrimetric determinations (aqueous and potentiometric). In addition to the above, elemental microanalysis is carried out. Where applicable,

optical rotation, optical rotatory dispersions, refractive index, functional group assay, the degree of hydration or solvolysis determinations and the preparation of derivatives are performed. Chromatographic procedures are most useful in the determination of the presence of a possible unidentified impurity. The combination of this procedure with the melting point, infrared and ultraviolet spectra is often sufficient for the analysis of a simple, stable, well-characterized compound. The determination of the purity of a more complex compound is often an analytical research problem requiring the use of a combination of sophisticated techniques, and requires taking into consideration the stability of the compound and its possible contaminants or decomposition products.

In addition to the identification and analysis of compounds under study, the analytical unit is deeply concerned with those physical and chemical characteristics of the compounds which must be considered in the handling, storage, formulation and administration of the material. Of increasing importance to the Program is solubility and stability data, which serves as a guide for drug formulation, storage, and shipping. In certain situations the decomposition of a compound, its solution or formulation may be followed by the observation of the ultraviolet absorption when the decomposition of the active moiety results in a change in absorption. For the stability of compounds which cannot be followed by this procedure, other methods must be devised. Among the other procedures used are chromatography, determination of halide ion concentration, nuclear magnetic resonance, and mass spectrometry. To validate the accuracy of these procedures, determinations are made on a sample to which known standards have been added. The analytical data on compounds of current interest is periodically reviewed and the data is assembled in the form of a Chemical Information Sheet. These Chemical Information Sheets are made available to the investigators using the material.

The need for the evaluation of the "shelf-life" of formulated drugs is an increasing problem. In order to establish the effective life of a drug, the continuing assay of these materials is required and is performed on a regular basis.

In addition, the necessary analytical data for the Food and Drug Administration are collected and assembled. Files are maintained on the drugs in clinical trial in compliance with the Food and Drug Administration regulations covering "Investigational New Drug Applications".

The Unit collects and files such data as:

1. The detailed method of preparation;

2. The supplier's analytical data sheet; and

3. The independent analytical evaluation of each lot of chemical or drug as evaluated by the Drug Development Branch directed quality control contracts.

The assembled data is of use also in writing purchase specifications for additional quantities of the compound. In general, attempts are made to keep specifications brief by listing those requirements which are judged to best

identify and assay the compounds.

Another function of the quality control group is the analytical evaluation and/or certification of materials purchased on the open marked. Inquiries received from scientific investigators and participating clinicians as to drug quality are also handled.

During the calendar year 1970, 86 compounds, representing 143 lots of bulk chemicals and 81 lots of clinical formulations, have been evaluated. Each of these evaluations is treated individually, and the extent of analysis for each sample is a matter that is jointly decided by the Drug Development Branch and the contractors. The analytical data occasionally indicates unacceptable materials resulting in their being returned to the manufacturer for reprocessing.

In addition, several special studies were performed. One analytical facility, Midwest Research Institute, determines the partition coefficient of compounds for use in structure-activity correlation studies. The procedure consists of equilibrating the compound in a mixture of n-octanol and water, and determining the concentration of the compound in each solvent. This often involves intensive analytical development in cases where the compound is relatively insoluble or highly soluble in one of the solvents.

Assistance was also extended to the formulation facilities with their analytical and formulation problems concerning compounds of interest to the Chemotherapy Program.

The Section directed two analytical-quality control contract laboratories. In addition to the two analytical laboratories, elemental analyses are obtained from Micro-Analysis, Inc., under a purchase order agreement.

TABLE VII

CONTRACT LABORATORIES FOR ANALYTICAL-QUALITY CONTROL

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Midwest Research Institute	Haggerty	NIH-69-2113
Stanford Research Institute	Lim	NIH-71-2070

Miscellaneous

One contract at Pomona College (NIH-69-2112) is for structure-activity studies. This contract is directed towards the use of the regression analysis techniques developed by Dr. C. Hansch of Pomona College to develop structure-activity correlations of antitumor agents and the accumulation of fundamental data relating to hydropholic bonding which will be used for further refinement of the structure-activity correlations.

During this report period a correlation was made for a group of nitrosoureas. The IP and IV L-1210 leukemia activity was found to correlate with the octanol-water partition coefficient (log P). While the correlations were not as good as one would like, all results did point in the same direction. That is, more hydrophilic nitrosoureas should be both more active and less toxic against tumors outside the brain. Based on these observations some nitrosoureas which would be more hydrophilic were designed and are being prepared. Work in this area of structure-activity correlations is continuing.

A preliminary evaluation of the hydrophilic - lipophilic characteristic of the compounds demonstrating activity in the L-1210 leukemia and the Walker 256 tumor systems was made. The preliminary study indicated that the Walker 256 test system selects more lipophilic compounds than does the L-1210 leukemia system. Work in this area of endeavor is continuing.

A contract at the University of Pittsburgh (PH43-67-1186) is for the determination of the structure via x-ray crystallographic techniques. The project is limited to the study of materials whose structures are otherwise difficult to establish and which are of significant interest to the Program. Thus, elucidation of new structures will permit comparisons with known active agents, allow establishment of structural criteria for antitumor activity, suggest unique synthetic departures in design of analogs and provide the basis for including selectivity in assays in drug quality control.

During this report period the contract has established the structures of three compounds. A nucleoside precursor, 6-chloro-9-(2'-deoxy-α-D-ribopyranosyl) purine diacetate, was found to exist in the α-glycosidic form rather that the β as had been determinded by less definitive methods. This important finding throws new light on stereoisomerism and antitumor activity in the nucleoside area.

Studies with the other two compounds (5-diazo-2'-deoxycyclouridine and 5-diazo-uracil) provided insight into their unexpected chemical inertness towards conversion to analogs of known active and clinically useful antitumor drugs. The x-ray determined structures suggested how the synthetic difficulties could be avoided. This information is now being used to implement laboratory syntheses of the desired analogs.

TABLE VIII

MISCELLANEOUS CONTRACT LABORATORIES

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
University of Pittsburgh	Abraham	PH43-67-1186
Pomona College	Smith	NIH-69-2112

CHEMICAL AND DRUG RECORDS SECTION

DRUG DEVELOPMENT BRANCH

Scope

The responsibilities of the Chemical and Drug Records Section are (1) to provide a continuous flow of chemical compounds for the primary screen and for other program needs (2) to develop, maintain and update systems of records keeping for all chemicals entering the program (3) to maintain adequate storage and inventory of bulk chemicals and drugs and (4) to coordinate the distribution of materials shipped to contract screeners and other interested investigators. These objectives are achieved through the concerted efforts by staff members to cultivate interest and to provide meaningful liaison with suppliers and potential suppliers; the surveilance of world-wide literature for new sources of chemicals and drugs; the development of chemical nomenclature for all compounds entering the program; the maintenance of Master Chemical Information files; the distribution and utilization of chemical data; the monitoring of contracts; and the insurance that all discreet compounds obtained from commercial sources are maintained in accordance with the signed agreements.

Staff

The Section is normally staffed with five professional chemists, one biologist and one secretary. Of the professional staff four are involved in accessioning of chemicals and drugs; the development of chemical information for input into the computer; and in chemical nomenclature. Two of these staff members are also concerned with the procurement of new synthetic chemicals and drugs for the primary screen. In addition to their assigned tasks the professional staff serves as project officers on various contracts that are maintained by the Drug Development Branch.

Acquisition of Compounds

The majority of compounds received by the Chemotherapy Program are contributed by commercial sources, universities and other non-profit organizations, government agencies, and interested individuals. To insure the adequate input of compounds, the Section is exploring ways to develop more effective means of monitoring the world-wide chemical literature for potential sources of new chemotherapeutic agents. This effort in literature surveilance will be expanded and will no doubt provide the program with many new types of compounds and drugs of interest. Through correspondence and by personal contact the staff provides information that often encourages the suppliers and potential suppliers to develop new areas of synthesis with the resulting preparation of new potentially active agents for testing. The success of the support from the suppliers is largely dependent upon the wide personal acquaintance of the Drug Development staff members with the scientific public, and the continued efforts by the staff towards good public relations. This significance of this conscientious effort to promote a mutually beneficial relationship is exemplified by the number of compounds the staff has collected from various research centers. Twenty

universities were visited during the calendar year. A total of 1566 compounds were collected of which 1219 were new structures. Continued efforts in being selective of the types of materials sampled has lowered the percentage of duplication for samples obtained from collection trips from 25% to 20%.

For the calendar year 1970 a total of 8521 crystalline compounds were accessioned of which 629 (7.4%) were prepared under contract; 247 (2.7%) were purchased; and 3037 (35.7%) were submitted by industry. Of the remaining 4608 (54.2%) compounds, 1219 resulted from collection trips and the balance of 3389 compounds entered the program through voluntary submissions from non-industrial sources. Included in this latter group are 1737 new compounds obtained from another government agency. It should be pointed out that during the six month period from July 1 through December 31 the overall percent duplication dropped to about 10%.

The total number of synthetic compounds registered by the Chemotherapy Program is approximately 160,000, a relatively small percentage when compared to 4 million compounds registered by Chemical Abstracts Services. The total number of ring systems which have been screened is approximately 1680. Of these, 251 have demonstrated activity and 5 new active ring systems were added in 1970.

Record Maintenance

A variety of records are maintained by the Section. These include Master Chemistry Card files, the Molecular Formula file for the purpose of compound data retrieval, the file of "Drug Abuse" compounds, and those records pertaining to inventory and distribution.

During the past calendar year significant steps were taken towards the development and implementation of a computerized chemical information retrieval system. In collaboration with the Progam Analysis Branch, the Section participated in many working sessions which culminated in the initiation of a mechanized approach for the identification of duplicates and for substructure search. The system utilizes procedures developed by the Chemical Abstracts Service (CAS), and the information is retrieved from the data bank of NCI registry of compounds.

A feasibility study for a total chemical information system was completed by CAS and steps are being taken currently for the design and implementation of such a system. Staff members have contributed extensively in time and effort in developing and adopting procedures to overcome many logistic problems which become apparent during the conversion from a manual search system to an automated one. Although many problems remain, they can be effectively resolved when the total system is implemented and brought in as an "in-house" operation.

The Section continues to provide excellent services in chemical nomenclature. The names assigned are based on systems developed by either Chemical Abstracts Services or International Union of Pure and Applied Chemistry. At the request of the Publication Section, Program Analysis Branch, the Section has reviewed the nomenclature of compounds in 35 articles approved for publication.

The Section is responsible for maintaining the confidential status of compounds received from industrial sources. The recognition by these organizations that the Branch maintains constant surveillance over commercial discreet materials has afforded not only a good industrial relationship but has made available many commercial samples not otherwise accessible. Frequently, there is need to obtain the release of compounds from confidential status. Declassification is achieved only when the industrial supplier agrees, in writing, to declassify his compound.

Drug Abuse Compounds

In compliance with the order issued by the Food and Drug Administration, the Section monitors and maintains records of input and distribution of materials considered by FDA as narcotics, barbiturates, hallucinogenic and other dangerous drugs. The FDA has specified that separate and accurate records be maintained for these materials and only those individuals with the proper narcotic license be approved to dispense and receive these items. To insure proper safety, the drug abuse compounds are secured in a separate locked file.

Chemical and Drug Distribution

The Chemical and Drug Records Section is also responsible for the receiving, storage and distribution of chemicals for screening. The Section is charged with the custody of bulk chemicals and drugs that enter the Program. Detailed inventory records, such as source, date received, amount, lot numbers, initiator of all shipments and destination of drugs, are recorded on a daily basis.

The Section monitors the portion of the contract (PH43-68-1283) with Microbiological Associates, Inc. which handles the receiving, weighing, record-keeping, distribution and storage of compounds. Over 140,000 different materials are held in storage. These consist largely of chemicals and to a lesser extent plant products, antibiotics and steroids. A total of 7695 new compounds were shipped to the screening laboratories; 6144 refills and materials for special testing were weighed and packaged for shipment. During 1970 approximately 14,000 samples were returned by the screening laboratories. These compounds, for which testing has been completed, are relogged into the inventory.

TABLE XIII

CONTRACTS MONITORED BY SECTION

CONTRACTOR	PROJECT OFFICER	CONTRACT NO.
Chemical Abstracts	Brown	PH43-65-1021*
Microbiological Associates, Inc.	Berman	PH43-68-1283

*Joint contract with Program Analysis Branch.

CLINICAL DRUG DISTRIBUTION SECTION

DRUG DEVELOPMENT BRANCH

Scope

The Clinical Drug Distribution Section is primarily responsible for the development, production, control, and distribution of all investigational dosage forms of interest to the Chemotherapy Program. The Section is also responsible for the procurement and distribution of commercial formulations used by the Chemotherapy Cooperative Groups.

The responsibilities of the Section can be categorized as follows:

1. Development of new dosage forms for clinical use;

2. Production and packaging of investigational drugs;

3. Procurement of commercial dosage forms;

4. Preparation of pharmaceutical data for FDA-IND submissions;

5. Quality control surveillance of all clinical dosage forms; and

6. Storage and distribution of clinical dosage forms.

Staff

The staffing of this Section consists of two pharmacists, two shipping and receiving clerks and one secretary.

Development of New Dosage Forms for Clinical Use

Formulation development commences when an agent is approved for toxicological study. The first approach to development involves an evaluation of existing chemical and physical data and a review of *in vivo* route and regimen studies in rodents. The accumulation and evaluation of these data are critical to the ultimate design of the dosage.

Developmental studies are assigned to one of the contract pharmaceutical laboratories. In preparing the assignment, the contractor is advised of the experimental data and desired dose forms.

Routinely, drugs considered for parenteral formulation are subjected to solubility and stability studies in a selected group of physiologically accepted vehicles. The drug solutions are then studied for the effects of p^H, temperature, light and atmospheric oxygen. Additionally, compatibility tests are performed with buffers, preservatives and vial closures. Drugs considered for oral formulation are studied for compression properties, compatibility with binders, photodegradation and temperature effects.

Some of the drugs recommended for development are either insoluble or poorly soluble in most commonly used physiological vehicles. Occasionally, these compounds are highly reactive and require special handling. These problems have been an obstacle in the preparation of some suitable parenteral dosage forms of potential antitumor agents. For this reason, the contract with the Pharmaceutical Research Department of the University of Michigan is designed for exploring and developing new concepts of parenteral formulation of drugs with limited solubility and/or stability.

Experimental formulations are submitted for evaluation and confirmation of antitumor activity. Simultaneously with these studies, the dosage forms are sent to an independent laboratory for chemical and/or biological analyses. The formulation data, screening and analysis results are evaluated by this Section for use by the Decision Network Committee. With committee approval, the dosage form is assigned for toxicological assessment in dogs and monkeys.

The toxicological results and the accumulated pharmaceutical development data are distributed to members of the Dose Formulation Committee. These data serve as a basis for determining the starting human dose and the ultimate dosage form to be used in the initial clinical studies. Several dosage strengths of the same investigational drug may be prepared provided the drug concentration remains within the development limits of the dosage form.

During 1970, formulation contractors developed 30 dosage forms, 19 of which were cleared for clinical trial.

Table XI illustrates the new dosage forms that were approved for clinical trials during 1970.

TABLE XI

NEW DOSAGE FORMS IN CLINICAL TRIAL

1970

NSC NO.	DRUG NAME	DOSAGE FORM
752	Thioguanine	75 mg/vial
1895	Guanazole	5 Gm/vial
10973E	Ethynyl estradiol	1 mg/tablet
18429	Aniline mustard	25 mg/capsule
71261	β-TGdR	10 mg and 50 mg/vial
79037	CCNU	40 mg and 100 mg/capsule
82196	Imidazole-4-carboxamide,5-{3,3-bis-(2-chloroethyl)-1-triazeno}-	100 mg and 300 mg/vial
95441	Methyl CCNU	20 mg and 100 mg/capsule
100880	Camptothecin Sodium	200 mg/vial
102627	1-Propanol,3,3'-iminodi, dimethanesulfonate (ester) hydrochloride	100 mg/vial
102816	5-Azacytidine	50 mg/vial

TABLE XI (Continued)

NSC NO.	DRUG NAME	DOSAGE FORM
107392	Picolinaldehyde,5-hydroxy-, thiosemicarbazone	100 mg and 500 mg/vial
406021	9H-Purine-6-thiol, 9-β-D-arabinofuranosyl	200 mg/vial
**409962	BCNU	100 mg/vial

**New method of preparation of 100 mg vials to provide for large scale production.

Production and Packaging of Investigational Drugs

This Section is responsible for the production, packaging and labelling of all investigational dosage forms used in the Chemotherapy Program. These functions are performed by:

1. NCI Formulation Contractors;

2. Other NCI Contractors with Formulation Capabilities;

3. Pharmaceutical Manufacturers not Contracted by NCI but having cooperative interests in a particular drug;

4. Direct purchase.

Most investigational dosage forms used in the Chemotherapy Program are produced by NCI formulation contractors. These contractors receive production specifications and guidance from this Section. The Clinical Drug Distribution Section monitored four development and production contracts during 1970 (Table XII).

TABLE XII

DRUG FORMULATION CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Ben Venue Laboratories, Inc.	Wickes	PH43-66-909
Paul B. Elder Company	Shank	NIH-69-2110*
National Drug Company	Scheindlin	NIH-70-2227
University of Michigan	Simonelli	PH43-66-1284

*Terminated October 31, 1970

Several contractors primarily engaged in bulk drug preparation have capabilities for dosage form production. These contractors are encouraged to develop and produce their own dosage forms. Specifications for formulation and production are provided to each contractor by this Section. This arrangement has proven economical, expedient, and extremely beneficial to the program. The following contractors participated in these activities during 1970:

CONTRACTOR	CONTRACT NO.
The Upjohn Company	PH43-68-1023
Bristol Laboratories	NIH-69-35
Merck & Company	PH43-62-479

Some dosage forms are developed and produced by pharmaceutical manufacturers on a cooperative basis. By such an agreement the manufacturer supplies the dosage form free of charge. These sources include several foreign drug manufacturers. The dosage forms are prepared from specifications which are established by mutual agreement between this Section and the manufacturer.

During 1970, the above pharmaceutical manufacturers supplied a total of 32,351 vials and 3,487 bottles of tablets and/or capsules to NCI.

Several investigational dosage forms are procured by direct purchase. Purchase requisitions and product specifications are prepared by this Section. During 1970, six investigational drugs were obtained by purchase requisition.

Procurement of Commercial Dosage Forms

The Section also obtains commercially available dosage forms for distribution to the Chemotherapy Cooperative Groups. These drugs are purchased either through the Veterans Administration Supply Catalogs, or by special purchase through negotiated contract mechanism. A total of 140,230 vials and 22,034 bottles of tablets and/or capsules were purchased during calendar year 1970.

All suppliers of commercial formulations are required to supply a certificate of analysis on each lot of drug purchased. The manufacturing and analytical data is reviewed by Staff prior to release of the formulation for use.

Quality Control of Clinical Dosage Forms

Each lot of formulated drug must meet specific quality control criteria prior to release for clinical use. This Section, maintains a strict quality control progrm designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., and oral products are tested for content uniformity, weight variation, hardness, disintegration, etc. All products are tested for potency by the formulator. Individual unit assays are required; the number of samples

assayed depend on the dosage form and the size of the production batch. All new dosage forms are routinely submitted to an analytical contractor to verify the acceptability of the product and the accuracy of the formulator. The confirmation analyses are mainly performed by the Stanford Research Institute and Midwest Research Institute.

This Section participates in the activities of the Quality Control Committee for Chemicals and Drugs. This committee reviews for clearance the analytical results of bulk chemicals and formulated clinical dosage forms.

During calendar year 1970, 202 lots of clinical dosage forms were reviewed by the Quality Control Committee for Chemicals and Drugs. Of these, 132 were investigational dosage forms and 70 were commercial dosage forms.

The Pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of the investigational dosage form. These studies are carried out under similated clinical conditions to test the effects of temperature, light and additives. The accumulated information is then reported on a Pharmaceutical Data Sheet which is incorporated into the Clinical Drug Brochure.

This Section also maintains a shelf-life surveillance program on all lots of noncommercial formulated dosage forms. Representative samples of each formulated lot are stored under specified controlled conditions and assayed for chemical and/or physical change. Two assay schedules are used for shelf-life studies: Schedule A, designed to provide stability data under accelerated conditions; and Schedule B, designed for controlled studies of repeat formulations where adequate stability data is available. Both schedules provide a two-year surveillance record.

Preparation of Pharmaceutical Data for FDA-IND Submissions

This Section prepares attachments I, II, III, V and VII for submission to Cancer Therapy Evaluation Branch for IND filing with the Food and Drug Administration. These attachments consist of the description composition, method of manufacture and controls used in the preparation of the dosage form. The FDA requires that such a submission be made for each new investigational drug prior to clinical release. In addition, IND amendments must be submitted whenever a new dosage form of an existing IND drug is formulated for clinical use.

During 1970, this Section prepared 11 original IND reports and 27 amended IND reports for submission to the FDA. Five IND's were filed during this report period (Table III).

Storage and Distribution of Clinical Dosage Forms

The receiving and shipping activities of the Section are carried out by two shipping clerks. These clerks have been instructed in the required handling techniques for each drug. They are knowledgeable of postal and freight procedures used in this country and throughout the world. A significant amount of clinical drugs are shipped to foreign investigators each year.

During 1970, approximately 460 foreign drug shipments were made.

During 1970, a total of 466,717 drug units (vials and bottles) were received and inventoried for subsequent world-wide distribution. During that same period, 430,380 drug units were distributed averaging 26 individual shipments daily.

This Section is also responsible for coding of drugs for double-blind studies. These studies may involve two or more drugs; the same drug in different dosage strengths, or a combination thereof. In all instances, these dosage forms are indistinguishable in appearance for double-blind purposes. The codes are placed on each bottle or vial by a supply clerk and checked by the pharmacist prior to shipment. There are presently 21 such coded studies on inventory.

Another phase of the Clinical Drug Distribution program involves the management of returned drugs from clinical investigators. Returned drug information is recorded on the original inventory file, including the name of the investigator, the amount of drug returned and the date received. This information completes the distribution cycle of the drug since the original shipping date is also on the same card by investigator's name, quantity shipped, etc. This Section is responsible for the disposition of the returned drug. The judgement to restock, destroy or quarantine a returned drug is dependent upon the condition of the drug container, the age and known stability of the dosage form, the amount of drug in question and other factors. There were 29,289 returned drug units recorded during this report period.

During 1970, the Section had an average inventory of 294 dosage forms by lot number involving 186 different formulations of 99 different compounds. These figures include investigational and commercial dosage forms.

An additional function of the Section is to provide drug distribution data to the Program Analysis Branch. This information has become significantly important for budgetary analyses of Cooperative Chemotherapy Groups.

A new shipping and receiving facility has been planned for the Section. It will provide additional floor space, greater low temperature storage area and a receiving platform. Construction is scheduled for completion by September 1971.

NATURAL PRODUCTS SECTION

DRUG DEVELOPMENT BRANCH

Scope

This Section has as its primary objective the development of novel cancer chemotherapeutic agents from microbial sources, higher plants and animal products. This area will be discussed under the headings of Fermentation Products Program and Plant and Animal Products Program.

Staff

This Section has operated with a staff of three persons, consisting of two professional (Ph.D. level) and one clerical employee.

Surveillance

World-wide surveillance of the published literature and collection of chemicals and drugs for biological evaluation are carried out in the Fermentation Products area. With the dropping of the plant literature survey project by the Program Analysis Branch last year and the inability to assign additional personnel to this work, it has not been possible to undertake surveillance in the Plant and Animal Products area.

Statistical Summary

Table I lists the natural product materials acquired during the year under review compared with previous years. Of the crude extracts, contractors provided practically all of the 2172 fermentation products, none of the 587 animal products, and 60% of the 3987 plant products.

Fermentation Products Program

Since the isolation of penicillin in the early 1940's, microorganisms have been an important source of new chemotherapeutic agents in the infectious disease area, and we believe they have as great a potential in cancer chemotherapy. Three groups of organisms have been shown to be the principal producers; they are the bacteria, actinomycetes, and fungi. Antibiotics produced by these microorganisms have shown a wide range of activity and of chemical structure and have introduced a number of new structure types to the chemical literature.

World wide, there have been 237 or more highly purified fermentation products reported to be active *in vivo* against one or more animal tumor systems. Of this group 124 have been entered in our program. Thirty-six of these have been in clinical study and 13 are still under clinical investigation. Those now in clinical study are Actinomycin D, Adriamycin, L-Asparaginase from *Escherichia coli*, Azaserine, Azotomycin, Bleomycin, Daunomycin, Mithramycin, Mitomycin C, Porfiromycin, Streptonigrin, Streptozotocin, and Tubercidin. Other fermentation products in clinical study in other parts of the world

include: Chromomycin A_3, Neocarzinostatin, Olivomycin A, Actinomycin C, and L-Asparaginase from Erwinia carotovora. All but Actinomycin C are under development in our program.

The antitumor fermentation products program has been carried out through contracts at a number of different biological laboratories, each of which used its own approach to soil collections, culture isolation, selection of culture types, fermentation development, and chemical isolation. Some of the contracts in this area have differed from the synthesis and plant product contracts in that a sizable amount of the effort was devoted to in-house screening and other assay services.

Earlier in our program we had a total of ten laboratories cooperating in the search for new agents. With reductions in available funds and changing priorities in some of the laboratories this is now reduced to three. These are listed in the accompanying Table IX. This reduction in program size has, of course, had a marked effect on the number of agents being fractionated and on the number of purified samples received for preclinical evaluation.

TABLE IX

CONTRACT LABORATORIES FOR FERMENTATION PRODUCTS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Bristol Laboratories	Dr. Bradner	PH43-69-35
Michigan Department of Health	Dr. Olson	PH43-65-7*
The Upjohn Company	Dr. Fonken	PH43-68-1023

*This contract provides a tissue culture assay laboratory for a fermentation program supported by the State of Michigan.

Emphasis continues on the screening of fungi though many actinomycetes are still included. During the year ten purified materials have been received for evaluation from the contract laboratories. One of these, B-153,797 K283, has demonstrated a high degree of activity in the Walker carcinosarcoma 256 (survival) over a wide range of doses.

Another one, NSC-141,537 (Anguidine), shows activity in both P388 lymphocytic (PS) and L-1210 lymphoid (LE) leukemias. It was described in Europe, and the English patent states that it causes a marked reduction in the leukocyte count in dogs. It has been reported that the leukosis is also seen in man. This is the first representative in our program of a very interesting new class of chemicals involved in toxicity to animals and man from cereal grains. The other samples received from our contracts are still under study.

The aureolic acid group of antibiotics continues to be of interest with Chromomycin A_2 (NSC-131,187) and Chromomycin A_3 (NSC-58514) being compared

on a number of different dose regimens. The Japanese dosage form of Chromomycin A_3 was obtained for toxicological studies.

The glutarimide antibiotics (such as Actidione, NSC-185, and E-73, NSC-32743) are receiving special study because of the report that E-73 crosses the blood-brain barrier. A summary report on this group was prepared and two new compounds of this type have been received for testing.

During the year, L-Asparaginase from Erwinia carotovora (NSC-106,977), mycophenolic acid (NSC-129,185) and the Merck Antibiotic (NSC-135,758) passed Decision Network 2. 5-Azacytidine (NSC-102,816), Bleomycin (NSC-125,066), Mycophenolic acid (NSC-129,185), and Adriamycin (NSC-123,127) have advanced beyond Decision Network 2 and have been cleared for initial clinical trials.

The fermentation and chemical literature continues to be scrutinized for new antibiotics or other metabolic products which should be screened against our in vivo systems. As a result, 38 purified samples and 73 crude samples were received for screening and a large collection of a lichen from Minnesota was received for extraction and screening. Seven fermentation products have passed Step III during the year. These comprise Actinomycins C_2 and C_3, Anguidine, Chitosan, Merck 593A, Orpheomycin, and Mycophenolic acid sodium salt. Four of these seven agents are from our contract program.

Plant and Animal Products Program

In carrying out its objectives in the plant and animal products area the Section has, in addition to the contract effort, developed programs with collaborating research grantees and other organizations in (1) plant and animal collecting; (2) preparation of extracts; (3) fractionation of active extracts and isolation and identification of the active agents, and (4) in collaboration with the Chemical and Drug Procurement Section in the preparation of large amounts of active agents for preclinical toxicology and clinical trial.

The search of the published literature for known constituents of active plants and attempts to acquire them for testing, mentioned in last year's report, have had to be dropped for lack of personnel.

The Section, through extensive correspondence, maintains contact with potential domestic and foreign suppliers in order to acquire plants, animals, and pure compounds of natural origin which are of special interest to the Program. In addition, this correspondence provides information relating to the use of plants and plant products in medicine (popular, native, and folklore).

Because of the increasing necessity in recent years to re-collect large amounts of natural products for fractionation, and the reduction in dollar amount of the contracts, the general collection of new plants under contract has substantially decreased. The normal level of approximately 5000 extracts per year has not been maintained even by seeking and obtaining new cost-free sources of supply. In the case of marine animals, most have been purchased from a variety of sources.

Progress was made in these areas of natural products, as follows:

A. Marine organisms

To date, confirmed active extracts have been obtained from 31 different marine organisms, 17 of which were obtained during 1970; 29 were active in P388 lymphocytic leukemia (PS) and 4 in cell culture (KB) (2 were active in both systems). These were representatives of the phyla Porifera (1), Coelenterata (6), Bryozoa (3), Echinodermata (5), Mollusca (8), Arthropoda (2), Chordata/Tunicata (4) and Chordata/Pisces (2). Of these, 11 species are under fractionation.

New commercial sources of these organisms have been developed and it is expected the future will see pure compounds of novel structure derived from this program.

B. Plants

During the year under review, the U.S. Department of Agriculture has initiated large-scale collecting projects with local organizations in South Africa, Taiwan and Panama which will yield us substantial numbers of new plants from each of these areas. Similar negotiations in other countries are also under way and should materialize in the near future.

A start has been made, with the U.S. Department of Agriculture, in analyzing our screening data and our chemical fractionation results with a view toward making the original plant collecting more productive. Already certain plant families have emerged as being good sources of novel and useful compounds while other families appear to be practically worthless. The U.S.D.A. botanists will be able to take advantage of these findings and plan their collecting to be more selective. For instance, Coniferae and Celastraceae appear to be rich in plant species yielding novel active compounds and a special effort is being made to concentrate on these families by planning collections in those localities of the world where they are known to be common.

During the year, all fractionators were instructed to discontinue working on all plants active in Walker carcinosarcoma 256 (WM), Sarcoma 180 (SA), Lewis Lung carcinoma (LL), Friend virus leukemia (FV), etc., which had not also been authorized for fractionation in LE, PS, or KB, and the U.S.D.A. was instructed not to re-collect such plants for fractionation purposes. This has resulted in a considerable shift of both collecting and fractionation effort in favor of those tumors in which we have the most interest, and should result in higher yields of interesting compounds in the future.

The following contractors are engaged in the Plant and Animal Products Program:

TABLE X

PLANT AND ANIMAL PRODUCTS CONTRACTORS

CONTRACTOR	INVESTIGATOR	CONTRACT NO.
Arizona, Univ. of	Brewer; Cole	PH43-67-1464
Bristol Labs. 1/	Bradner	PH43-69-35
Central Drug Res. Inst.	Dhar	01-004-1 (PL-480)
Costa Rica, Univ. of	Saenz R.	PH43-64-31
Hawaii, Univ. of	Sagawa	PH43-67-748
Midwest Res. Inst. 1/	Cheng	PH43-65-94
Research Triangle Inst.	Wall	NIH-69-2019
Southern Res. Inst. 1/	Montgomery	PH-43-64-51
U.S. Department of Agriculture	Perdue	NCI-CR-64-1 (Transfer)
Virginia, Univ. of	Kupchan	PH43-64-551
WARF Institute, Inc.	Derse	PH43-67-747

1/ Minor part of the total contract.

Beside the contractors listed above, certain major investigational groups have been recruited and contribute to the Plant and Animal Products Program without cost to CCNSC, as follows:

1. The University of Brazil, Rio de Janeiro, has been collecting native Brazilian plants and preparing the corresponding extracts for screening by CCNSC. Recently, a few extracts from marine invertebrates have been provided.

2. Since its contract terminated in Calendar 1968, the Commonwealth Scientific and Industrial Research Organization (C.S.I.R.O.), Melbourne, Australia, has been fractionating several native plants from among the more than 100 that have shown reproducible antitumor activity. C.S.I.R.O. has not been able to fractionate all these plants in its own laboratories and has arranged to have some of the work done by 17 collaborators in

Australian university laboratories and other government laboratories.

3. Mr. Martin Jacobson, Pesticide Chemicals Research Branch, U.S. Department of Agriculture, Beltsville, Maryland, is fractionating several active plants under the guidance provided by our bioassays. Two of the insect extracts, recently provided, have proved to be active and one of these is under fractionation.

4. The Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Illinois, is also fractionating several active plants under the guidance of CCNSC bioassays. Cephalotaxus, in particular, has yielded several novel alkaloids of which one, harringtonine (NSC-124,147), has demonstrated high activity in P388 Leukemia.

5. Dr. P. R. Enslin, Council for Scientific and Industrial Research, Union of South Africa, is fractionating 7 active plants and is re-collecting several others for fractionation. These plants are from South Africa and originally arose out of the Smith Kline and French work. During the year under review, arrangements have been made for the C.S.I.R. to collect native plants more systematically and in larger numbers and to prepare extracts for submittal to CCNSC.

6. Dr. P. R. Jefferies, Univ. of Western Australia, working independently of the C.S.I.R.O., supplied native plants for routine screening and is fractionating 7 active ones.

7. Dr. G. R. Pettit, Arizona State University, has been heavily engaged in preparing and fractionating animal extracts (insects and marine forms) for submittal to the Program and has begun to isolate some of the active agents. He is also collecting and extracting plants (387 this year) and is fractionating 5 of the confirmed active ones.

8. Dr. Norman R. Farnsworth, University of Illinois, has supplied us with many plant extracts. He is currently fractionating 13 of the active ones.

9. Dr. J. M. Cassady, Purdue University, supplies us with many plants, mostly from Indiana, and has four of them under fractionation.

10. Dr. M.-L. King, National Defense Medical Center, Taiwan, supplies us with extracts of many Taiwan plants. Two of them are under fractionation.

11. Dr. A. J. Charlson, University of Natal, So. Africa, has supplied us with some native plants and is currently fractionating two of them.

12. Several NIH research grants are devoted to the isolation of native products from plants and to the study of their pharmacological properties. In some cases, CCNSC performs the bioassays necessary to guide the fractionation of these plants. Recently, several grantees have shown interest in fractionating active plants supplied to them by CCNSC. Among those actively engaged in fractionating plants supplied by us are Dr. R. W. Doskotch, Ohio State Univ.; Dr. David Kingston, State University

of New York at Albany; Dr. Mario Silva, Concepcion, Chile; Dr. J. L. Wong, Univ. of Louisville; and Dr. L. H. Zalkow, Georgia Institute of Technology.

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ANNUAL REPORT

DRUG EVALUATION BRANCH

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER

CHEMOTHERAPY
NATIONAL CANCER INSTITUTE

Principal objectives of the Drug Evaluation Branch (DEB) are to initiate, develop, and coordinate laboratory programs for recognition, recommendation, and development of new drug therapies against human cancer. Objectives are achieved by (a) screening a large number of synthetic and natural products for biological activities suggesting clinical usefulness, (b) evaluating new "active" drugs and older drugs with respect to conditions of usage (e.g., treatment route and schedule, and combination chemotherapy) which may enhance therapeutic efficacy leading to cures of animal tumors, (c) studying drug effects on host immune responses and their implications to therapy, (d) investigating mechanisms of drug action and resistance, (e) developing improved methods for drug evaluation, and (f) investigating problems relative to drug evaluation arising during preclinical toxicological examination, clinical formulation development, and clinical trial.

DEB includes the Office of the Chief (J. M. Venditti) and three sections; Biochemistry (F. R. White, Head), Immunotherapy and Immune Response Modifiers (IIRM, J. P. Glynn, Head), and Screening (B. J. Abbott, Head) and is staffed by 11 senior professional (including two CO's and two SA's), four junior professional and technical, and five statistical and clerical personnel. Program is implemented through contracts with qualified institutions and in intramural laboratories in collaboration with other intramural and extramural projects. DEB staff have primary administrative responsibility for 12 Chemotherapy contracts, and 4 contracts under the Special Virus Cancer Program (Etiology), and in addition, monitor scientific and technical tasks related to drug evaluation under 15 additional Chemotherapy contracts and one Carcinogenesis (Etiology) contract. Chemotherapy Program tasks monitored by DEB staff are denoted by IIC; III A, B, C; VD; and VIIA, B, D in Tables I-III of the Report of the Scientific Director for Chemotherapy. Comprehensive drug evaluation programs were continued at Southern Research Institute (71-2098), Arthur D. Little, Inc., ADL (65-61), and Microbiological Associates, Inc., MAI (68-1283), where tasks were devoted principally to detailed evaluation of drugs in development toward clinical trial, the conduct of therapeutic trials in tumor-bearing animals leading to cures, and the development of new screening assays. Tasks conducted under DEB sponsored contracts with Southern Research (71-2098 as well as 66-29 for the study of biochemical mechanisms) continued to be integrated with Southern Research projects related to pharmacological distribution of active drugs (65-594), the pharmacology of drug combinations (70-2083), and the synthesis of new potential antitumor drugs (71-2021). The Drug Evaluation Division and Immunology Division, MAI, represent collaborative laboratory resources for DEB staff who participate in an advisory capacity in day-to-day planning and evaluation of tasks (see Individual Project Reports, Serial Nos. NCI-3576 and 3577). In addition, the

Screening Section and IIRM Section include modest in-house basic and developmental research programs (see Individual Project Reports, Serial Nos. NCI-3577 and 3578). Full implementation of projected in-house laboratory programs are still hampered by a lack of sufficient professional and technical research personnel. Currently, the work is conducted by two technicians (one for each section), Staff Associates, and part-time employees.

During calendar year 1970, 15,155 synthetic and natural products were submitted to CCNSC for determination of antitumor activity in primary screens. One hundred thirty five showed "confirmed activity" in initial in vivo screening stages; that is, they reproducibly met minimal criteria for activity at a high level of statistical significance. Such agents were subjected to further testing, and levels of activity substantially greater than the minimal end-points for "activity" were required for recommendation as a clinical candidate. With continual accumulation of both laboratory and clinical results, criteria for recommendation as a clinical candidate continued to be reviewed and modified. The Drug Evaluation Committee reviewed screening results for 238 agents and recommended 6 to Chemotherapy Staff as candidates for development toward clinical trial. Seven agents passed Decision Network 2 (DN-2) of the Chemotherapy Program "Linear Array". These and their bases for selection are listed in the current organization report of the Screening Section.

L1210 leukemia remained the basic assay for routine primary screening of all new agents using two treatment schedules, single treatment followed by daily treatment. The single treatment schedule had been added to the L1210 protocol in 1966 to (a) permit screening using at least two widely divergent schedules in response to an increasing awareness of the importance of scheduling to drug efficacy, (b) provide a basis for selection of doses for daily treatment, and (c) permit some in vivo screening of synthetic materials submitted in limited quantity. The latter objective was realized as evidenced by a marked increase in the percentage of submitted synthetics that are actually being screened in vivo. While the value of single treatment relative to economy of drug usage suggests that it should be retained for materials in limited supply, it has not been a highly productive schedule for uncovering new drugs and it has not been as effective as anticipated for predicting dosage levels for daily treatment. Comprehensive analysis of the relative effectiveness of daily treatment, single treatment, and intermittent treatment for 100 "L1210-active" drugs, showed that among these schedules, considered as feasible for primary screening, daily treatment was the most productive in uncovering L1210-activity at the minimal end-point, 25% increase in lifespan (ILS); whereas intermittent treatment was the most productive in uncovering activity at the 50% ILS level, generally regarded as the minimal effect required for a recommendation as a clinical candidate. Accordingly, the L1210 screening protocol was modified within the past year to provide for the continued use of daily treatment whenever possible and the use of intermittent treatment in preference over single treatment for materials received in moderately limited amounts. Crude natural products were screened in vitro to provide an inexpensive bioassay for the fractionation of those that demonstrated in vivo activity. Synthetic agents were screened in vitro only if the supply was insufficient for in vivo screening.

CCNSC protocols for screening were last published in 1962 and revised since by specific instruction to screening laboratories as required. These protocols have now been updated to describe current CCNSC practice and are in preparation for publication. While the previously published protocols described methods for 21 in vivo systems, the new protocols will include methods for the five in vivo tumors in current use; mouse leukemias L1210 and P388, mouse melanotic melanoma B16, mouse Lewis lung carcinoma (LL), and rat Walker 256 carcinosarcoma. P388 and L1210 are used routinely for in vivo screening of crude natural products. The rationale for the use of both L1210 and P388 for in vivo screening of natural products was based on previously reported findings that these tumors tend to respond to the same kinds of agents but that P388 is quantitatively more sensitive to materials that are active against both. Concisely, it was felt that potential L1210 activity might be "masked" in L1210 screening of a crude plant extract or fermentation beer but might be uncovered and isolated using the more sensitive P388. Analysis of screening results against L1210 and P388 for plant products since the commencement of this practice is underway. Because of the time required for recollection and fractionation of plant products, relatively few P388 actives have been isolated to a point permitting L1210 testing of a relatively pure material. Analysis of relative P388 and L1210 activity for 113 materials derived from antibiotic filtrates showed that at the 25% ILS level, 55 were inactive against both, 29 were active against P388 only, and 29 were active against both. Of the latter, 11 were active against L1210 at the 50% ILS level. Of these 11, eight showed greater activity against P388 than against L1210. Eighteen materials were active against P388 at the 50% ILS level but the activity against L1210 fell between 25 and 49%. The possibility remains that schedule dependency studies might uncover L1210 activity at the 50% ILS level (cf. the uncovering of the schedule dependent L1210 activity of Emetine, NSC-33669). A recent analysis of comparative data for 83 agents of current or previous clinical interest showed that of 31 with an ILS $\geq$ 100% against P388, 19 exhibited an ILS% $\geq$ 50% against L1210. Of 20 exhibiting an ILS between 50-99% against P388, 10 attained an ILS $\geq$ 50% against L1210. Of 32 producing an ILS $<$ 50% against P388, only six were active at the 50% level against L1210.

Testing against IM-W256 continued to be restricted to agents in some stage of development based on activity in this system. Active IM-W256 agents were tested against the IP-W256 tumor for their effect on survival. The IP-W256 system was used to study relative antitumor activity among a number of dihydrofolate reductase inhibitory folic acid analogs which are bound in mouse serum and tend to be inactive against mouse tumors but which are not bound in rat or human serum. One such W256 active agent, NSC-113423 (Baker's antifol) was selected as a clinical candidate. Work with these agents is continuing in the search for a more soluble agent with similar biochemical, pharmacological, and antitumor activity (see current organization report, Biochemistry Section).

Studies of the biological characteristics, kinetics of proliferation, and response to therapy of B16 and LL continued. Drugs of clinical interest were evaluated in both systems to provide information on their spectrum of sensitivity to such agents. Hopefully, an anticipated increase in screening capacity will permit the utilization of one or both of these tumors as an

ancillary in vivo screen. The current report of the Screening Section compares the response of L1210, P388, B16, and LL to a number of agents. The drug sensitivity of B16 appeared to be conditioned by the site of tumor implantation prompting study of the characteristics and kinetics of growth of the tumor implanted IP or SC at ADL and Southern Research, respectively. Preliminary efforts were made to determine the relationship between the size of SC, IC, and IV implants and host death or tumor growth. Results indicated that SC-B16 growth is typically Gompertzian, that the S-phase of 500 mg. tumors is about 1/3 of the cycle time, metastasis occurs following implantation, and this system may be manipulated to provide a quantitative assay for viable tumor cells surviving treatment. Studies of the kinetics of IP-inoculated B16 proliferation (ADL) indicated that the S-phase on day 9 after implantation is about ½ the cycle time. Kinetic studies of SC-implanted LL (Southern Research) indicated that the S-phase of 500 mg. tumors is nearly ½ the cell cycle time. Summary results of studies of the growth kinetics of selected experimental tumors of interest are shown in the current report of the Screening Section. The use of either B16 or LL for the screening of new agents and detailed evaluation of drugs emerging from other screens is predicated on the hypothesis that such tumors may select agents which are not primarily inhibitors of DNA synthesis. Available data suggest that either tumor might fulfill this need. Either SC-B16 or LL appears to be utilizable for surgical-adjuvant studies, but LL may be more suitable for this purpose. Following SC or IM implantation, LL metastasis to the lung is predictable. MAI is attempting to quantitate the extent of lung metastasis following IM-LL implant and to relate therapeutic activity to effects against the local tumor at the implant site and against the metastatic lesions. Southern Research is studying the kinetics of metastatic proliferation and exploring models for surgical-adjuvant studies against LL.

In vitro studies of drug effects against specific stages of the cell life cycle were related to the search for drugs with a high degree of selective toxicity to tumor cells but which do not depend on S-phase inhibition. The Upjohn (68-1023) group have studied a number of drugs for their comparative cell kill potential against synchronous and asynchronous populations of DON cells in culture. Exponential cell kill of asynchronous cells was attributed to non-cell cycle stage specific inhibition. Drugs whose cytotoxicity to asynchronous cells reached a saturation level were considered to be cell cycle stage specific, and these were studied against synchronized cells to elucidate the stage of the cell cycle inhibited. Such studies confirmed the S-phase inhibition by agents known to be specific DNA inhibitors but also suggested that certain agents (e.g., Act. D, NSC-3053; Nogalamycin, NSC-70845; and Daunomycin, NSC-82151) inhibit specifically but in late G_1 or early S-phase, and that Neocarzinostatin (NSC-69856) may be a specific G_1 inhibitor. Recently, a contract (71-56) was negotiated with Dr. Tobey of the U. S. Atomic Energy Commission (Los Alamos) to pursue further the determination of the extent to which drugs may inhibit viable cells in G_1 arrest. The latter studies are aimed at the development of an experimental model based on the contractor's established method for the reversible G_1 arrest of cultured Chinese hamster cells and the release of arrested cells permitting nearly 100% progression into S-phase. Should this method prove feasible for screening, its use in larger scale contract projects is anticipated.

Studies of the biological characteristics and response to chemotherapy of AKR lymphoma continued as a collaborative project among the IIRM Section and the Screening Section, DEB and with the Southern Research Institute. All drugs in recent or current development toward clinical trial have been evaluated against primary AKR lymphoma. In addition, a number have been examined for their ability to maintain remissions of primary disease induced by combination therapy with Vincristine (VCR, NSC-67574) plus prednisone (NSC-10023E) or dexamethasone. An assay for drug evaluation against virus-induced primary leukemia in BALB/C mice has been developed. It is anticipated that the primary AKR lymphoma and the primary BALB/C leukemias will continue to be used to study selected drugs and combinations of drugs for their ability to induce or maintain remissions. However, drug evaluation tests against these primary leukemias are costly in terms of material and personnel resources. Thus, these systems are not feasible for the screening of large numbers of new agents. If they are to be used to uncover new agents, it will be necessary to employ a suitable and feasible pre-screen for selecting candidates for trial against the primary disease. Evaluation of current information on the kinetics of proliferation of first generation transplanted, long-passaged, and primary AKR lymphoma and their responses to known agents suggests that long-passaged lines are similar to L1210 with respect to growth characteristics and drug sensitivity. In contrast, first generation transplanted AKR, while kinetically similar to L1210 for a short time after implantation, displays growth characteristics in the advanced state similar to those of the primary tumor. Therapy with a limited number of agents suggest that the spectrum of drug sensitivity for the advanced first generation tumor is comparable to that of the primary tumor. For example, cyclophosphamide (NSC-26271) cured a large percentage of mice with early first generation tumor, and substantially increased lifespan but did not generally cure mice with advanced first generation or the primary tumor. Cytosine arabinoside (Ara-C, NSC-63878) cured mice with early first generation tumor but was only minimally effective in increasing lifespan of mice with advanced first generation or primary tumor. Methotrexate (MTX, NSC-740) increased the lifespan of mice with early first generation tumor but was ineffective against advanced first generation tumor and the primary tumor. However, it is not clear that these relative activity relationships will obtain for a large number of drugs. In studies of 14 additional drugs conducted in our intramural labs, six (BIC, NSC-82196; the methane sulfonate derivative, NSC-102627; 5-FU, NSC-19893; BCNU, NSC-409962; 5-Azacytidine, NSC-102816; and Isophosphamide, NSC-109724) were active (ILS $\geq$ 50%) against both the early first generation transplant and the primary tumor. Four (Camptothecin, NSC-100880; Emetine; VCR; and Ellipticine, NSC-71795) were active against the first generation tumor and inactive against the primary tumor. Four were inactive against both (Cis-Pt II, NSC-119875; Prednisone; DIC, NSC-45388; and Gallium nitrate, NSC-15200). It should be emphasized that these studies have been limited with respect to the dosage levels and treatment schedules used because of the limitation on available AKR mice until recently. The relative inactivity of VCR alone or Prednisone alone against the primary tumor is most interesting in view of the ability of these drugs in combination to produce 60-70% remissions of this disease. In order to obtain a substantial base of data on the potential use of the first generation transplantable tumor as a pre-screen for candidates for evaluation against the primary disease, a project is being developed to have one of our primary screeners test a reasonable number of materials against early first

generation transplantable AKR. Active agents will be evaluated against the advanced disease and active agents from the latter will be evaluated against the primary tumor.

A comprehensive study of treatment schedule dependency among 60 drugs of clinical interest because of activity against early mouse leukemia L1210 showed that these agents could be broadly grouped as follows: those which failed to exhibit schedule dependency (e.g., 6-MP, NSC-755; DIC; and 5-Azacytidine); those which were schedule dependent for one treatment on day 1 only (e.g., Cyclophosphamide; Isophosphamide; BCNU; and CCNU, NSC-79037); for divided treatments on day 1 only (e.g., Ellipticine; and Adriamycin, NSC-123127); for intermittent treatment giving either one injection or divided injections on each treatment day (e.g., 2-amino-1,3,4-thiadiazole, NSC-4728; NSC-102627; and ICRF-159, NSC-129943); for intermittent treatment giving one injection per treatment day (e.g., MTX; Emetine; Neocarzinostatin; and Camptothecin); for divided-intermittent treatment (e.g., Azaserine, NSC-742; Guanazole, NSC-1895; Hydroxyurea, NSC-32065; Ara-C; 5-HP, NSC-107392; and the epipodophyllotoxin derivative, NSC-122819); and those that were schedule dependent for daily treatment (e.g., Procarbazine, NSC-77213 and Streptozotocin, NSC-85998). The study led to the following general conclusions. Schedule dependency cannot be predicted on the basis of chemical structure, and close structural analogs do not necessarily exhibit similar patterns of schedule dependency. Thus, when analogs of known clinically useful drugs emerge as active from the primary screen, they should be examined for schedule dependency regardless of their degree of screening activity relative to the parent compound. Schedule dependency via the oral route cannot be predicted from parenteral schedule dependency tests. In this program, the oral effect of an active drug is determined very early in its development. When formulation development studies reflect a poor prognosis for clinical parenteral usage of an orally active drug, oral schedule dependency is studied in detail. The extent of disease at the time of treatment initiation can alter activity and schedule dependency as well, suggesting that drugs destined for clinical trial should be examined against both advanced and early tumors. Schedule dependency of bulk compounds in suspension may be altered for their soluble clinical formulations. The latter, as they are developed, are examined for schedule dependency.

Empirical results of treatment schedule studies when related to the kinetics of tumor and normal cell proliferation, intracellular drug actions, and the influence of drug concentration and duration of effect suggested that the pattern of schedule dependency exhibited by a drug may be related to the interval between injections within one course of treatment (in the L1210 model, the number of injections on each treatment day) and to the larger interval between courses (in the L1210 model, the interval between the days of treatment). An advantage to divided treatments over a single injection per treatment day was attributable to one or more of a number of factors including (a) the total cell population, (b) the drug's cell cycle specificity of action (a consequence of its biochemical site of action) relative to the kinetics of tumor and critical normal cell proliferation, and (c) its rate of excretion, metabolic inactivation, or activation. Extension of the interval between days of treatment enhanced the effectiveness of a number of drugs characterized by varying degrees of specificity of S-phase inhibition

suggesting that the optimal interval between treatment courses is mainly a function of the time required for host recovery from toxicity. The importance of considering biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules for active drugs was emphasized by the Southern Research group who utilized such data to predict the divided-intermittent treatment schedule dependency of guanazole, hydroxyurea, and 5-HP. Further, these investigators related biochemical sites of inhibition to patterns of resistance and cross-resistance among DNA inhibitors and showed that an L1210 variant selected for resistance to the DNA polymerase inhibitor, Ara-C, retained sensitivity to the ribonucleotide reductase inhibitors, Guanazole, Hydroxyurea, and 5-HP. Moreover, L1210 variants selected for resistance to any of the ribonucleotide reductase inhibitors exhibited cross-resistance to the others (despite their structural dissimilarity) but retained sensitivity to Ara-C. Such results prompted the study of combinations of Ara-C plus the individual ribonucleotide reductase inhibitors against the parent L1210 tumor with the finding that, in each case, the combination was more effective than either Ara-C alone or the ribonucleotide reductase inhibitor alone.

Following the previously reported anti-L1210 synergism between 6-thioguanine (NSC-752, 6-TG) and Ara-C given concomitantly (q3 hrs., q4 days), more detailed study of this combination at Southern Research showed that simultaneous administration of the two agents doubled the tolerated dose of 6-TG. However, sequential use of these agents (Ara-C followed by 6-TG) resulted in enhanced toxicity and little therapeutic advantage. This observation prompted studies of the influence of the sequence of administration on the therapeutic activity of drug combinations against L1210. For example, while Ara-C followed by CCNU provided a therapeutic advantage over treatment with either drug alone, CCNU followed by Ara-C resulted in increased toxicity and loss of therapeutic efficacy. Similar observations attended the use of Ara-C followed by Cyclophosphamide and the reverse sequence.

In additional studies of the anti-L1210 effectiveness of drug combinations conducted at Southern Research, MAI, and ADL, the following drug pairs were observed to be more effective than either drug alone. In general, the drugs were combined using the optimal treatment schedule for each.

DIC + Melphalan (NSC-8806)
DIC + Ara-C
Cyclophosphamide + Camptothecin
Cis-Pt II (NSC-119875) + the quinolinium derivative, NSC-113089
Cis-PT II + Isophosphamide
Cis-Pt II + ICRF-159

Combinations of hydroxyurea + DIC or 6-TG, 6-TG plus 5-HP, BCNU plus VCR, and L-asparagirase plus azaserine were not more effective than individual drug therapy against L1210. Guanine failed to potentiate the toxicity or anti-tumor (L1210) activity of a number of adenosine analogs.

The therapeutic synergism of DIC plus Ara-C (MAI) was noteworthy inasmuch as parallel bioassay studies showed that Ara-C resistance develops in L1210 after three q3 hr., q4d. combination treatments. Thus, leukemic mice treated with the combination failed subsequently to respond to Ara-C alone but did respond to DIC, MTX, BCNU, and BCNU plus MTX. The study was important because it described a feasible L1210 model for evaluating subsequent therapy with a third drug following "remission induction" with an effective combination. Studies of Cis-Pt (II) in combination with ICRF-159 or cyclophosphamide in mice that had been inoculated with various L1210 cell concentrations provided models for the quantitation of the relative leukemic cell destructive capacity of combination and individual drug therapy (MAI).

ADL continued to study combinations of L-asparaginase (NSC-109229) plus other antileukemic drugs against mouse leukemia 5178Y. Combinations of L-asparaginase plus Daunomycin, MTX, Ethionine (NSC-751), VCR, Showdomycin (NSC-93047), or Prednisone were no more effective than L-asparaginase alone. Combinations of L-asparaginase plus azaserine, Duazomycin A, Azotomycin, Actinomycin D, or 6-mercaptopurine were more effective than any of the individual drugs. BCNU plus L-asparaginase presented no advantage over BCNU alone treatment of the L-asparaginase resistant subline of L5178Y.

The Southern Research group, in studying drug effects against established tumors, found that Bleomycin (NSC-125066) plus MeCCNU (NSC-95441) was not clearly more effective than MeCCNU alone against LL, but the combination did appear to provide a potentiative effect against SC-B16. MAI found that marked additive lethality to normal mice was not incurred when VCR, cyclophosphamide, Prednisone, or nitrogen mustard was followed by Bleomycin treatment.

The search for more effective folic acid antagonists focused on the previously discussed analogs of NSC-113423, and Dr. Bertino's (Yale Univ., 67-1193) studies to determine the effectiveness of other folate congeners as inhibitors of dihydrofolate reductase from L1210 cells, human CML, and human erythrocytes from MTX treated patients. Representatives of two classes of antifols, quinazolines and substituted triazines were as effective or more effective than MTX as enzyme inhibitors (see current report, Biochemistry Section).

Southern Research (66-29) continued to study mechanisms of action of 8-azainosine (NSC-130279). Its site of action has not been determined but it does not appear to inhibit sites attacked by other purine analogs. The finding that adenosine carboxylate (NSC-103526) inhibited GMP kinase has prompted the search for additional inhibitors of this enzyme among adenosine analogs. An opened ring aldehyde was identified as another metabolite of cyclophosphamide. The metabolite, a product of microsomal enzyme activity, inhibited H. Ep. 2 clone formation and its cytotoxicity was decreased by aldehyde oxidase. Continuation of studies of the mechanism of action of nitrosoureas suggested that their biological activity was associated with alkylating function to a large extent but not entirely. Other factors related to nitrosourea activity are under study.

Dr. Alton Meister (Cornell Univ. Medical College, 69-60) continued to study L-asparaginase (NSC-109229). L-asparaginase from agouti serum has been purified 800-fold with an 80% yield. However, the purified enzyme is quite unstable and until greater stability is achieved, further isolation of the enzyme system depleted by L-asparaginase, it was shown that ornithine decarboxylase increased markedly in RADA1 cells in vitro at a time when the cell number increased only slightly, and that L-asparaginase prevented both the increase in enzyme activity and subsequent cellular proliferation. L-aspartate was found to be necessary for the asparagine synthetase catalyzed exchange of inorganic pyrophosphate and adenosine triphosphate and that the reaction proceeded through β-L-aspartyladenylate. The search for specific inhibitors of asparagine synthetase with antitumor activity was continued without success and a more random selection of agents from those submitted to CCNSC will be studied (see current report, Biochemistry Section). Dr. John Wriston (Univ. of Delaware, 69-2115) has achieved the isolation of L-glutaminase free from L-asparaginase activity. Upon its purification, about 200 mg. of the enzyme should be available for antitumor testing.

At the Weizmann Institute of Science (PL-480 agreement), Dr. Katchalski continued the study of the ability of low molecular weight polypeptides to penetrate bacterial cell walls. It was found that free amino acids or four-residue polypeptides penetrated readily but that polypeptides with 5 or more amino acid residues did not. Ornithine peptides were not hydrolyzed and polymers of this positively charged amino acid can cover negatively charged cell membranes suggesting their use as carriers for various functional groups. Poly-DL-alanine which remains outside the cell membrane was used to study the mechanism of permease mediated lactate transport into cells. The permease was shown to take up lactose on the outside of the membrane, rotate, and release it at the inner surface. Concanavalin A (NSC-143504), a water soluble lectin isolated from plant seeds, was shown by Katchalski to bind to specific cell membrane sugars. The bivalent metals of Con A are required for sugar binding. Substitution of $^{63}Ni^{++}$ for the native Ca^{++} or Mn^{++} of Con A and determinations of cell-bound radioactivity showed that the number of Con A binding sites increases 3-5 times when cells in culture are treated with trypsin, or transformed by virus, chemical carcinogen, or x-ray. Small lymphocyte cellular division was stimulated by Con A. It appeared that, in order to stimulate mitosis, Con A must be in constant contact with the cell membrane. When it was removed by α-methyl-mannoside prior to mitosis, division was not stimulated. Dr. Bruce (Toronto) has reported informally that Con A agglutinated a mouse fibrosarcoma and we have sent him the Lewis lung tumor for similar studies. Southern Research is also studying the effects of Con A against LL. DEB in-house laboratory studies have shown Con A to be ineffective against L1210 but moderately effective against P388.

Proposed Course:

Primary screening in vivo of all agents against leukemia L1210 and of natural products against leukemia P388 as well will be continued. In addition, the in vivo screen will be expanded to provide for the screening of as many new agents as possible against a slow-growing transplantable tumor (e.g., Lewis lung carcinoma or B16 melanoma) and against spontaneous tumors (e.g., AKR lymphoma). Continued emphasis will be placed on the detailed study of active

agents with respect to the achievement of cures in animals by determining maximally effective treatment regimens and the use of drug combinations. Continuation of efforts to integrate schedule dependency, cytokinetic, biochemical, and physiological disposition findings will be aimed at the prediction of optimal conditions for usage of active drugs. Greater emphasis will be placed on the achievement of cures using all available therapeutic means including chemotherapy combined with surgery, radiation, or immunotherapy. Studies of mechanisms of action of active drugs will be broadened to the investigation of biochemical sites other than those directly related to nucleic acid biosynthesis. The cellular functions inhibited by highly cytotoxic agents which have not demonstrated antitumor specificity in limited in vivo primary screening will be explored. In the latter connection, it is anticipated that routine in vitro screening of synthetic agents will be reinstituted. Thus, DEB plans to continue both intramural and contract supported studies aimed directly at the selection and development of improved therapies for trial against human cancer and to pursue basic research and developmental tasks which supplement or complement this principal goal.

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Serial No. NCI-3576
1. Chemotherapy, CCNSC
2. Drug Evaluation Branch (DEB)
3. Office of the Chief
4. Microbiological Associates, Inc. (MAI)
Cancer Chemotherapy Department
Drug Evaluation Division
Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1970 - June 30, 1971

Project Title: Detailed Evaluation of Potential Antitumor Drugs.

Previous Serial Number: Same

Principal Investigator: John M. Venditti

Co-Principal Investigators: A. Goldin, B. J. Abbott (NIH), I. Kline, V. S. Waravdekar, R. J. Woodman, M. Gang, A. Sirica, R. B. Field (MAI, contract no. PH-43-68-1283).

The Drug Evaluation Division, MAI, is a collaborative laboratory resource for the research staff, DEB, which acts in an advisory capacity in the day-to-day planning, execution, and evaluation of tasks.

Man Years (NIH): Total 1, Professional 1

Project Description: This is a multifaceted project devoted to the development of new potential antitumor drugs.

Objectives:

To (1) evaluate new synthetic and natural products in experimental systems designed to uncover antitumor drugs; (2) study in detail the antitumor actions of drugs which emerge as active from primary screens; (3) provide information applicable to toxicological, pharmacological, and biochemical study and to clinical use of new antitumor drugs; (4) provide information on optimal conditions for therapeutic efficacy in animals (route and schedule dependency, combination chemotherapy, development of resistance, etc.) leading to cures; and (5) conduct basic research in the development of improved assays for drug evaluation.

Methods Employed:

Studies are conducted mainly in rodents with transplantable tumors (leukemia L1210, drug-resistant variants of L1210, Lewis Lung carcinoma) employing

established procedures and new procedures developed in the Drug Evaluation and Chemotherapy Divisions of this project and within DEB, CCNSC.

Detailed descriptions of accomplishments under this project are contained in the annual and interim progress reports, contract PH-43-68-1283.

Drugs discussed in this project report are listed below with their NSC numbers and abbreviations used.

NSC No.	Name
740	MTX; Methotrexate
746	Urethane; Carbamic acid, ethyl ester
1895	Guanazole
3070E	Diethylstilbestrol; 4,4'-Stilbenediol, α,α'-diethyl-
4728	2-Amino-1,3,4-thiadiazole
9848	Phenobarbital; Barbituric acid, 5-ethyl-5-phenyl-
19893	5-FU; 5-Fluorouracil
25154	Piperazine, 1,4-bis(3-bromopropionyl)-
26271	Cytoxan; Cyclophosphamide
27640	5-FUdR; 5-Fluorodeoxyuridine
32065	Hydroxyurea
33669	Emetine
39690	SKF-525A
45388	DIC; 5-(3,3-dimethyl-triazeno)-imidazole-4-carboxamide
46401	α-Sarcin
49842	VLB; Vinblastine
52695	Cytoxyl alcohol; phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-(3-hydroxypropyl)-, cyclohexylamine salt
56054	Pseudourea, 2,2'-(9,10-anthrylenedimethylene)bis-2-thio-, 2HCl
58514	Toyomycin; Chromomycin A3
63878	Ara-C; Cytosine arabinoside
66020	2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-5-pyrrol-2-yl-
69856	Neocarzinostatin, crystalline antibiotic
71795	Ellipticine
74841	Ethylenediamine, N-(2-chloroethyl)-, diHCl
75520	5-F_3TDR; Uridine, 2'-deoxy-5-(trifluoromethyl)-
77213	Procarbazine, p-Toluamide, N-Isopropyl-α-(2-methylhydrazino)-p-toluamide, hydrochloride
79037	CCNU; 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea
83265	3-Tritylthio-L-alanine
91728	Urea, 1,3-bis(2-fluoroethyl)-1-nitroso-
92188	Isoquinaldaldehyde, thiosemicarbazone
95441	Me-CCNU; 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
100880	Camptothecin, sodium salt; Indolizino[1,2-b]quinoline-7-glycolic acid, α-ethyl-9,11-dihydro-8-(hydroxymethyl)-9-oxo-,sodium salt
102627	1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester, HCl)
102722	Urea, 1,3-diallyl-
102816	5-Azacytidine; s-Triazin-2(1H)-one, 4-amino-1-β-D-ribofuranosyl-
104800	DBD; Dibromodulcitol
106767	Urea, 1-(2-fluoroethyl)-1-nitroso-3-(tetrahydrothiopyran-4-yl)-, S,S-dioxide

NSC No.	Name
107392	5H-Pyrazone, thiosemicarbazone
109723	2-[Bis(2-chloroethyl)amino]-3-(2-chloroethyl)-tetrahydro-1,3,2-oxazaphosphorine, 2-oxide
109724	3-(2-chloroethyl)-2-[(2-chloroethyl)amino]-tetrahydro-1,3,2-oxazaphosphorine, 2-oxide
113089	6-amino-1-ethyl-4-[p[[p-(4-pyridylamino)-phenyl]carbamoyl] anilino]quinolinium, ethobromide
113233	Mitomalcin
114347	6-amino-4-[3-amino-4-[[p-(4-pyridylamino)-phenyl]carbamoyl] anilino]-1-methyl-quinolinium,p-toluene sulfonate, metho-p-toluenesulfonate
118994	α-(hydroxy-methyl)-α'-(6-hydroxy-9H-purin-9-yl)diglycoaldehyde
119875	cis-Platinum (II); Platinum, diamminedichloro-, cis-
123127	Adriamycin
125066	Bleomycin
127439	Chitosan chloride
129943	ICRF 159; 4,4'-Propylenedi-2,6-(±)-piperazinedione
135057	Thioethylenedi ara-C; urea, 1,3-bis(1-β-D-arabinofuranosyl-1,2-dihydro-2-oxo-4-pyrimidinyl)-2-thio-
526051	α-Toluenesulfonic acid, α-anilino-, sodium salt

Major Findings:

I. Screening and Related Methodology.

Attention continued to be directed to developing an improved and simplified Lewis Lung Carcinoma (LL) assay system capable of detecting drugs effective against slow-growing tumors which encompass 80% of clinical tumors and are relatively recalcitrant to prevailing drug therapy. The LL assay system, modified to permit concomitant evaluation of tumor inhibition and increase in survival was studied for (a) reproducibility of results, (b) criteria for quality control, (c) relationship between tumor inhibition and survival, and (d) criteria for antitumor activity.

From among over 70 synthetics studied, selected from agents which are either markedly effective, moderately effective or relatively ineffective against leukemia L1210, 22 agents elicited inhibition of tumor and/or increase in survival and studies are in progress for confirmation of activity and for the optimal drug route and schedule of treatment. Such agents will also be studied for their capacity to inhibit LL metastases. Hopefully, agents will be characterized relative to their ability to inhibit primary tumor, inhibit LL metastases and increase survival.

Evaluation of LL activity of four S-phase cell cycle dependent agents, 5H-pyrazone, thiosemicarbazone (NSC-107392), ara-C (NSC-63878) hydroxyurea (NSC-32065) and guanazole (NSC-1895), given every 3 hours on every 4th day compared with one treatment daily are in progress.

An additional 124 plant product fractions which had passed the original LL routine screen were reexamined. As another resource when needed for the CCNSC L1210 screening program, 1600 new synthetics were screened. A new derivative (NSC-135057) of ara-C, synthesized by the Organic Chemistry Division, MAI, was considerably more effective given as a single treatment than a single ara-C treatment.

II. Agents Effective against Lewis Lung.

A. Nitrosoureas. The five nitrosoureas studied, BCNU (NSC-409962), Me-CCNU (NSC-95441), CCNU (NSC-79037), NSC-106767, and NSC-91728 demonstrated marked activity with a single treatment.

B. Cytoxan and the Two Analogs, NSC-109723 and NSC-109724. Cytoxan was consistently effective given as a single treatment against the early or late stage of the disease. Of the two analogs tested on single treatment, NSC-109724, the more novel structure, was superior to NSC-109723 and comparable to cytoxan.

C. Other Effective Agents. The following additional agents elicited either (a) >75% TWI and >125% ILS, (b) >75% TWI and <125% ILS, or (c) >75% TWI and <125% ILS: NSC-125066, 46401, 107392, 4728, 3070E, 27640, 25154, 526051, 32065, 127439, 746, 92188, 74841, and 118994.

III. Other Studies with Effective LL Agents.

A. Me-CCNU. Two treatments 9 days apart or three treatments 7 days apart did not provide an advantage over one treatment.

B. Cytoxan analog, NSC-109724. Daily, weekly or twice weekly treatments were not superior to treatment on one day. This observation applies also to the parent compound, cytoxan.

C. Bleomycin (NSC-125066). Daily treatment with this agent appears to be superior to a single treatment or intermittent chronic treatment.

D. ICRF-159. Although this agent elicited a moderate inhibitory effect against the primary tumor, preliminary studies employing the Wexler assay indicate that it markedly inhibits the development of LL pulmonary metastases.

IV. Activity of Effective L1210 Agents Administered by a Route other than the Site of Implantation of Tumor Cells.

After much expense for clinical preparations it was found that mitomalcin (NSC-113233) is active only when administered IP against the IP tumor. To avoid the possibility of this situation arising, drugs considered for clinical formulation are studied for IP and SC activity against SC implanted ascitic L1210. Drugs active SC against early SC L1210 will be tested SC against advanced SC-inoculated L1210. Out of 24 agents of clinical or of potential clinical interest which have been tested IP and SC against early SC L1210, 15 agents retained their activity: NSC-1895, 25154, 33669, 45388,

66020, 77213, 83265, 100880, 102816, 109723, 109724, 113089, 114347, 119875, 129943. Four agents were ineffective or displayed reduced activity against the SC tumor: NSC-52695, 58514, 69856, and 123127, and five agents displayed somewhat reduced activity: NSC-56054, 71795, 102627, 104800, and 107392; these agents are currently being retested for confirmation of results.

V. Other Studies with New or Old Drugs.

A. Vinblastine (NSC-49842). This agent does not appear to be schedule-dependent in the early L1210 or early P388 tumor systems. Treatment in divided doses was moderately superior to treatment with a single injection per day in mice with early P388 tumor, but provided no advantage in mice with early L1210.

B. 5-F_3TDR (NSC-75520) and 5-FU (NSC-19893). 5-F_3TDR did not display antileukemic activity superior to 5-FU. Multiple injections per day with 5-F_3TDR were superior to one injection per day given daily or intermittently. With 5-FU, however, multiple injections per day did not provide an advantage.

C. Me-CCNU (NSC-95441). In mice with early leukemia, this agent is markedly effective over a wide range of treatment schedules and is effective given IP, SC, or orally. The optimal daily dose was generally lethal to non-leukemic mice and daily treatment for 5 or 9 days was the least effective schedule. None of the treatment schedules employed were superior to treatment Q3H on day 1 only.

D. NSC-113089 and NSC-114347. These quinoliniums which have demonstrated marked activity whether given IP or SC against IP or SC tumor were further examined to compare the activity of formulations prepared by heating the materials with formulations prepared at room temperature. NSC-113089 was soluble whether heated or prepared at room temperature and the antileukemic activity of the two formulations was comparable. NSC-114347 was in solution when heated and in suspension when prepared at room temperature. The antileukemic effectiveness was comparable, but considerably higher doses were required for the materials in suspension.

E. NSC-45388. Previously reported *in vivo* tests, which showed that SKF-525A extended the biological half-life of cytoxan and phenobarbital decreases it prompted investigations on the influence of SKF-525A and phenobarbital, which respectively retarded and accelerated the action of liver microsomal enzymes, on the efficacy of NSC-45388 (DIC), an *in vivo* enzymatic activated drug. Two tests showed that SKF-525A elicited a minimal reduction in host toxicity of DIC given orally and phenobarbital, on the other hand, produced a striking increase in host toxicity. The maximum increase in survival was comparable whether DIC was given alone or after treatment with SKF-525A or phenobarbital.

VI. Combination Chemotherapy of L1210.

Thirteen two-drug combinations were tested for the first time or explored in greater depth to determine the optimal therapeutic benefits from combination drug treatment. A number of diverse experimental conditions were used. The following drug combinations provided increases in lifespan greater than those produced by the individual drugs:

NSC-26271 + NSC-100880
NSC-26271 + NSC-113089
NSC-26271 + NSC-119875
NSC-45388 + NSC-63878
NSC-109724 + NSC-119875
NSC-119875 + NSC-129943

A. NSC-45388 (DIC) + NSC-63878 (Ara-C). This combination in five experiments elicited increases in lifespan superior to those produced by optimal single drug therapy. Parallel bioassay studies showed that ara-C resistance develops after three Q3H/24H treatments on every 4th day suggesting that the rapid onset of resistance to ara-C limited the benefits from the combination. Of particular interest was the observation that animals treated with the combination from day 5 to day 13 or 17 did not respond to subsequent treatment with ara-C alone, but did respond to treatment with DIC, MTX, BCNU, or BCNU plus MTX, agents effective against the L1210 variant resistant to ara-C. The studies show that the rapid development of resistance to ara-C may limit the therapeutic benefits of this combination and indicate the usefulness of sequential therapy with other drugs in enhancing the therapeutic advantage of this combination.

B. ICRF-159 (NSC-129943)+ cis-Platinum (II) (NSC-119875). Combination treatment with two new antileukemic agents, ICRF-159, a derivative of the chelating agent, versene, and cis-Pt-II, a novel structure for an antileukemic agent, was superior to the individual drugs given alone against both the early and late stage of L1210. The therapeutic advantage of this combination of agents with interesting structures may be associated with unusual, but unknown, mechanisms of antitumor action.

C. Cis-Platinum (II) + Cytoxan. Day 1 combination therapy of mice implanted with a wide range of inoculum levels was superior to the single drugs. For example, combination treatment produced 100% 60-day survivors against the $3.3x10^5$ inoculum compared with 25% and 12% by cytoxan or cis-Pt-II alone. In two studies, a single combination treatment with these agents on day 3 elicited a greater percentage of long-term survivors as compared with either drug alone. Against day 5 L1210, combination therapy did not yield survivors, but did elicit superior increases in survival compared with cytoxan or cis-Pt-II alone. The combination of cis-platinum (II) with the cytoxan analog, NSC-109724, was superior to single drug treatment but not superior to the combination with the parent agent, cytoxan.

D. Other Combination Studies. Studies combining a set of compounds of particular interest to the chemotherapy program with cytoxan (which reportedly acts without regard to a cell's cycle) and with ara-C (which reportedly acts only during the S-phase of the cell cycle) are in progress.

VII. Intracerebrally-Implanted L1210.

This IC L1210 assay system has been useful for studying the ability of drugs to penetrate the "blood-brain" barrier. In the current tests, the following drugs were studied intracerebrally:

NSC-129943, ICRF-159
NSC-119875, cis-Platinum (II)
NSC-77213, MIH
NSC-102722, Urea, 1,3-diallyl
NSC-104800, DBD
NSC-109723, Cytoxan analog
NSC-109724, Cytoxan analog

ICRF-159 was studied in combination with two agents of demonstrated activity against this system, BCNU and ara-C.

A. ICRF-159 Alone and in Combination. It was suggested that this relatively non-polar drug might pass the "blood-brain" barrier. In five experiments with the IC L1210 this agent produced increases in survival which were not greatly below the increase elicited against IP L1210. Treatment Q3H on every 4th day, the optimal schedule against IP L1210 may be the maximally effective schedules against the IC implanted L1210. A single treatment with BCNU on day 3 plus intermittent treatment with ICRF-159 was not superior to BCNU treatment alone. Combination treatment every 4 days with ICRF-159 plus ara-C Q3H every 4 days produced a greater increase in lifespan than treatment with ara-C alone.

B. Other Agents. The two cytoxan analogs elicited increases in lifespan comparable to the parent compound, cytoxan, an agent known not to cross the "blood-brain" barrier in mice. MIH and DBD produced a moderate increase in survival. Urea, 1,3-diallyl and cis-platinum were ineffective.

VIII. Development of an L1210 Variant Resistant to Cytoxan. Previously developed cytoxan resistant sublines of L1210 were partially sensitive to cytoxan. The current L1210-Cyt/R ascites is apparently fully resistant to cytoxan and may provide an additional useful tool for studying related alkylating agents and agents of diverse structures.

Proposed Future Course:

Quantitative evaluation of drugs of potential clinical interest will continue as indicated under "Objectives". Tasks related to activities within the Linear Array of the NCI-Chemotherapy Program's convergence format will be emphasized along with research and development tasks designed to supplement, complement, replace, or improve such activities. Activities will be carried out in collaboration with the DEB intramural laboratories.

Publications:

See current report, Drug Evaluation Branch, Office of the Chief.

BIOCHEMISTRY SECTION

DRUG EVALUATION BRANCH

Objective

The objective of the Biochemistry Section is to explore biochemical approaches to the improvement of therapy of human cancer. This may be accomplished by studies of the mechanism of action and development of resistance to agents of known anticancer activity, or through comparison of metabolic patterns of tumor and normal tissues so that new agents may be identified or designed to exploit differences found. The work summarized in this report was conducted under contract as indicated, or in collaboration with an NIH grantee (B. R. Baker). Aside from the diverse tasks performed by Southern Research Institute (Contract PH43-66-29), the effort was directed toward individual agents or classes of agents.

Accomplishments During the Past Year

Folic Acid Antagonists

Dr. J. R. Bertino (Yale University School of Medicine; contract PH43-67-1193) determined the activity of antifols submitted to CCNSC as inhibitors of partially purified dihydrofolic reductase from mouse leukemia L1210, human chronic myelogenous leukemia (CML), and human "induced" erythrocytes (from methotrexate-treated patients without hematological disease). Agents of two classes, quinazoline antifols (Parke-Davis) and substituted triazines (B. R. Baker), were as effective or more effective than methotrexate (MTX) in inhibiting the enzymes. In cooperative studies, NIH grantee Dr. B. R. Baker (University of California at Santa Barbara) used 2,2-dimethyl-4, 6-diamino-s-triazine as an active-site directing group to which various side chains were attached to confer specificity. The compounds inhibited dihydrofolate reductase, some at concentrations lower than MTX. It was found that these agents: 1) enter cells by passive diffusion rather than active transport as with folic acid analogs, 2) are bound to some constituent in mouse serum but not at all to human and only slightly to rat serums, 3) are excreted unchanged in the urine unless the side chain contains a sulfonyl fluoride group ($-SO_2F$) which is oxidized to sulfonic acid, and 4) may cross the blood-brain barrier.

Last year one of Dr. Baker's compounds (NSC-113423) was selected as a candidate for clinical trial, but presented a problem in formulation because of low solubility (1.1 mg/ml H_2O). Dr. Baker has modified the molecule in numerous ways to give solubilities up to 100 mg/ml of water. In vivo testing of the soluble congeners leading to the selection of a substitute for NSC-113423 is nearly complete.

L-Asparaginase (NSC-109229)

Dr. Alton Meister (Cornell Medical College, contract NIH-69-60) continued studies of L-asparaginase in four areas.

1) Agouti serum enzyme was purified 800 fold (80% yield, 10.9 I.U./mg protein) by using an affinity column of D-asparagine bound to Sepharose. However, the purified enzyme was unstable. Activity diminished rapidly in frozen solution and was nearly completely lost by lyophilization. Until the stability problem is solved, the eight liters of agouti serum now on hand, in which the enzyme is stable, will not be processed.

2) In a search for the enzyme(s) critical for cell maintenance or division which asparaginase depletes, ornithine decarboxylate activity in RADA1 cells was investigated in vitro. Activity increased rapidly in early growth, reaching a maximum at 12 hrs. when there was only a slight increase in cell number. At 36 hrs. when the cell number was maximum, there was virtually no enzyme present. Asparaginase prevented the rise in both ornithine decarboxylate and cell number.

3) L-Aspartate was found to be a requirement for the reaction catalyzed by asparagine synthetase, in which the net result is the exchange of inorganic pyrophosphate and adenosine triphosphate. Synthesis of β-L-aspartyladenylate established that the reaction proceeded through this postulated intermediate. Not only was the amount of ATP formed proportional to the amount of the intermediate, but each further addition of β-L-aspartyladenylate resulted in an immediate increase in ATP formation.

4) The search for inhibitors of asparagine synthetase has progressed slowly, and no inhibitors with in vivo antitumor activity have been found. A more random approach, which will utilize selected agents from the CCNSC collection will be examined.

SOUTHERN RESEARCH INSTITUTE CONTRACT PH43-66-29

Studies of the mechanism of anti-L1210 and anti-Ca755 action of 8-azainosine (NSC-130279) have not defined its specific site of action. However, most sites at which other purine analogs act have been eliminated, thus marking this agent as a promising candidate for combination therapy as well as for use by itself. The limited supply of the drug is a major problem.

Because the carbocyclic analog of adenosine (NSC-103526) was found to be a potent inhibitor of GMP kinase (the first inhibitor of this enzyme identified), the search for other inhibitors among adenosine analogs is underway.

Sublines of L1210 resistant to the ribonucleotide reductase inhibitors, hydroxyurea, guanazole, and thiosemicarbazones were developed. The subline developed for resistance to each ribnucleotide reductase inhibitor displayed cross-resistance to the others but retained sensitivity to cytosine arabinoside (Ara-C), a DNA polymerase inhibitor. An Ara-C resistant subline of L1210 was sensitive to the ribonucleotide reductase inhibitors. With the ribonucleotide reductase inhibitors, inhibition of DNA synthesis paralleled cytotoxicity. These observations provide a biochemical basis for combining Ara-C and ribonucleotide reductase inhibitors in cancer chemotherapy, and such combinations

displayed potentiation of activity against the parent L1210 line.

This year another metabolite of cyclophosphamide, postulated to be an aldehyde of the opened ring, was prepared by incubation of the drug with mouse liver microsomes. Its activity in inhibition of H. Ep. 2 clone formation was about double that of nitrogen mustard. Oxidation of the microsome-formed intermediate by purified aldehyde oxidase greatly decreased its cytotoxicity.

In further attempts to define the mechanism of action of nitrosoureas, C^{14} labeled BCNU was found in the protein fraction of treated L1210 cells. The identity of the labeled constitutent from nucleohistone with the product of 2-chloroethylisocyanate and lysine was established. From a study of the alkylating activity of a series of nitrosoureas by the p-nitrobenzylpyridine assay it was concluded that the biological activity depended upon alkylating activity to a considerable extent, but that other factors such as lipid solubility and reactivity of the generated isocyanates as well as steric factors were also of importance.

Cell cycle time analyses were made of two spontaneous tumors, AKR mouse lymphoma and DMBA-induced mammary tumors in rats. In both all phases of the cell cycle times fell within the limits observed for the many other tumors which have been studied, but real differences were observed in thymidine indices. The proliferative fraction of AKR leukemia cells was between 8% and 30% in contrast to nearly 100% for L1210 cells. The thymidine index of transplanted DMBA induced mammary tumors was three times that of the primary induced tumor.

Studies of the cytokinetic effects of useful anticancer agents (Ara-C, hydroxyurea, guanazole, MTX, 5FUdR, 6MP, 6MeMPR, etc.) were correlated with physiological disposition (concentration X time) studies conducted under other contract projects at Southern Research leading to more precise definition of factors influencing the optimal usage of these agents, alone and in combination, to maximize their therapeutic efficacy.

WEIZMANN INSTITUTE OF SCIENCE (PL-480 - Agreement No. NIH-06-025-1)

Among low molecular weight peptides, those up to four amino acids in length as well as the free amino acid were found to penetrate bacterial cells, but penta- and peptides higher did not enter the cells unless degraded into peptides with four or less amino acid residues. Peptides of ornithine were not hydrolyzed. Polymers of this positively charged amino acid were found to cover cells with negatively charged membranes and could act as carriers of other desired groups such as fluorescent labels, etc.

Much recent work with amino acid polymers emphasized their potential use as probes to study cell membranes. A mixed polymer of leucine and ornithine was found to agglutinate SV40-transformed cells of many types, but not the same cells when they were untransformed or polyoma infected.

A poly DL-alanine, which also remains outside cells, was used to study the mechanism of permease mediated lactose transport into cells. The lac permease

was shown to take up lactose at the outer surface of the cell membrane, rotate, and release it at the inner surface. The permease contained an SH group which was necessary for its activity, and which could be poisoned by mercury. The polymer, with mercury attached on very short side chains inactivated half the permease (that with the functional group at the outer surface), but if the mercury was attached by longer side chains it inactivated 100% of the permease (that functioning at the inner membrane surface as well). Similarly SH groups attached to the polymer by short and long side chains "rescued" half and all respectively, of previously "poisoned" permease.

Concanavalin A is one of about a dozen water-soluble lectins isolated from various plant seeds. Each binds to specific sugar(s) associated with cell membranes. For example, Con-A binds to mannose and glucose, and phytohemagglutenin (PHA), another lectin, to galactose, etc. Bivalent metals (native constituents of Con-A) are necessary for its sugar-binding property. Katchalski's group has substituted $^{63}Ni^{++}$ for the usual Ca^{++} or Mn^{++}. By counting the cell-bound radio-activity of $^{63}Ni^{++}$, the number of binding sites on each cell was found to increase from 3-5 times when cells in cultures treated with trypsin or transformed by viruses, chemical carcinogens, or x-rays.

Small lymphocytes treated with Con-A or PHA, are stimulated to divide. It was found that Con-A, to effectively trigger mitosis, must be bound continuously to the cell membrane. When it was removed by α-methyl mannoside prior to mitosis, cellular division was not stimulated.

Proposed Course:

It is anticipated that studies related to the mechanism of action of active antitumor agents will be broadened during the next year and will include the effects of such agents on cellular functions other than those directly related to nucleic and biosynthesis. In addition, the important question of the factors controlling the passage of a cell from the G_1 phase to the S-phase of the cell cycle will be investigated in detail. An *ad hoc* group of investigators is being formed to advise on pertinent studies related to this problem. Institution of broader programs for screening at the biochemical level, especially with highly cytotoxic agents which have not shown antitumor specificity in limited *in vivo* screens, is being explored.

Publications:

See current report, Office of the Chief, Drug Evaluation Branch.

IMMUNOTHERAPY AND IMMUNE RESPONSE MODIFIERS SECTION
DRUG EVALUATION BRANCH

Principal objectives of this section are (1) develop and utilize assay systems for detection of tumor-specific antigens, (2) study the role of tumor specific or associated antigen in the neoplastic process, (3) determine whether antibodies and immunocytes from animals immunized with preparations containing tumor-specific antigens retard or enhance development of authochthonous or transplanted tumors, (4) develop and utilize methods to evaluate the combined with immunotherapy, (5) develop and utilize methods to evaluate the immuno-depressive and immuno-enhancing properties of new and established drugs, (6) conduct appropriate investigations of resistance to anti-tumor agents which might be mediated via immune phenomena, (7) conduct basic and developmental studies with spontaneous and induced primary leukemias leading to the development of new assay systems for potential anti-tumor agents, (8) develop and provide information on tumor-host systems relative to their use in the CCNSC screening program. Significant findings are summarized in Individual Project Report, Serial No. NCI-3577.

The Section Head, as project officer, member, and vice chairman of the Special Animal Leukemia Ecology Segment of the Special Virus Cancer Program, shares the responsibility for the program and individually monitors four contracts. At the Albert Einstein Medical Center in New York (PH43-65-612), Dr. Frank Lilly is studying the genetics of susceptability of virus-induced leukemia and the biological and chemical properties of the virus-induced transplantation antigens. The group at the University of Pennsylvania School of Veterinary Medicine (PH43-65-1013) under Dr. Robert Marshak is investigating the etiology of bovine lympho-sarcoma. Dr. Charles Rickard of the School of Veterinary Medicine, Cornell University (PH43-65-620) is studying the etiology of feline leukemia. Dr. Michael Sigel, University of Miami (PH43-67-118) is attempting to develop procedures for the isolation and characterization of the virus-induced tumor-associated transplantation antigens of fowl leukosis. Significant findings are summarized in the Report of the Special Virus Cancer Program, Viral Oncology, Etiology.

Serial No. NCI-3577
1. Chemotherapy, CCNSC
2. Drug Evaluation Branch
3. Immunotherapy and Immune Response Modifiers Section
4. Microbiological Associates, Inc. (MAI), Immunology Division, Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Transplantation, Serological, Chemotherapeutic and Immune-Chemotherapeutic Studies with Murine Leukemias and Sarcoma

Previous Serial No.: Same

Principal Investigator: J. P. Glynn

Other Investigators: A. Goldberg

Cooperating Units: Screening Section (B. J. Abbott) and Office of the Chief, Drug Evaluation Branch (J. M. Venditti), Experimental Therapeutics (D. A. Cooney), NCI;MAI (M. Kende)

Man Years (NIH):

Total: 3
Professional: 2
Other: 1
Plus animal care support from Mammalian Genetics and Animal Production Section, CCNSC.

Project Description:

Objectives:

1. To investigate tumor-associated antigens and host reactivity to such antigens aimed at utilizing the knowledge derived from both the development of immuno-therapeutic and immune-chemotherapeutic models for mouse leukemia.

2. To determine whether antibodies and immunocytes from animals immunized with preparations containing tumor-specific antigens either retard or enhance the development of autochthonous or transplanted tumors.

3. To develop and utilize systems to evaluate the immuno-depressive and immuno-enhancing properties of new and established drugs.

4. To conduct basic and developmental studies with spontaneous and induced primary leukemia leading both to the development of new assay systems and the acquisition of sufficient data to use such models for combined immuno-chemotherapy.

Methods Employed: Standard Laboratory procedures were employed.

Major Findings:

I. Immunization With Tissue Culture Growth Cells

A. Transformed 3T3 Mouse Fibroblasts: Our most recent immunization method of sensitizing spleen cell donors requires injection of live, infectious (competent) Moloney sarcoma virus. Although this type of immunization is highly successful in providing spleen cells capable of eradicating transplantable lymphoma, some of the long term survivors developed primary sarcoma. This prompted us to use mouse fibroblasts that have been transformed by murine sarcoma virus. These cells are negative for leukemia virus but harbor Sarcoma virus genome and therefore might have the transplantation antigen. This line was developed and supplied to us by Dr. R. Bassin, NCI, Viral Leukemia and Lymphoma Branch.

CDF_1 (Balb/c x C57Bl/6)F_1 mice were immunized with transformed 3T3 mouse fibroblasts. The immunizing cell suspension was free of competent sarcoma as well as leukemia virus. Two weeks after the second immunizing dose the mice were challenged with graded amounts of transplantable lymphoma (LSTRA). A moderate degree of transplantation resistance was obtained. In the immunized group 80% of the mice were alive and tumor free at day 185 following the tumor challenge while in the control only 20% did as well. In a similar experiment 40% of the immunized mice survived 120 days vs none for the control. Serum samples from immunized and non-immunized mice were tested for the presence of MRF neutralizing antibody using the mixed culture CPE test of Rowe et al. No antibody was present above the low level found in normal sera. The transformed and the normal 3T3 did not absorb neutralizing antibody from known anti-MSV immune mouse serum. Immunization with four cell doses did not increase the transplantation resistance. In one test the normal 3T3 line also evoked some transplantation resistance, but in a repeated test using the normal cell line, normal CDF_1 and NIH Swiss mice spleen-suspension as immunogens, no transplantation resistance was seen.

B. Immunization With Non-producer Rat Neoplastic Cells (MSB-1) Harboring Defective Moloney Sarcoma Virus: MSB-1 line (epethetial neoplasma) was induced by MSV in thymectomized BN rats. The extracts of tumors and cultured cells consistently failed to induce tumors in newborn mice and did not induce foci on mouse embryo cells, nor did MSB-1 cells react in the CF test with broadly reactive sera of rats immunized with leukemogenic virus.

MSB-1 cells were supplied by Dr. R. Ting. Cells were harvested after 3-4 days of incubation. Two doses of ~5 x 10^6 cells/mice were inoculated IP two weeks apart. Two weeks later 10^5 - 10^3 LSTRA challenge cells were inoculated SC. Marginal transplantation resistance was obtained in the first test, which was not repeatable in a second test. In the third experiment four immunizing doses spaced every two weeks did not result in transplantation resistance.

C. Immunization Against AKR Leukemia: AKR-mice have been reported to be naturally tolerant to the virus associated antigen carried on AKR tumor cells and therefore the problem may be to find a suitable strain of animals that can be effectively immunized and serve as spleen donors for immunotherapy.

Attempts to immunize (AKR x DBA/2) F_1 were unsuccessful. Preliminary data of other laboratories suggest that (AKR x C57Bl/6) F_1 (B_6AK) can be immunized to AKR leukemia.

Attempts were made to immunize B_6AK with x-irradiated AKR leukemic spleen cells. The mice were immunized with two doses of 10^7 cells two weeks apart and two weeks later challenged with transplantable AKR leukemia. No immunity was observed.

Further attempts in which the immunogen was changed were also successful. Trocar implanted fragments of C58 gross virus induced tumor were also used for immunization of B_6AK. The mice were then challenged with thymus cells from AKR mice with spontaneous leukemia and no protection was found.

The tissue culture growth neoplastic Osborn-Mendel rat kidney cell line was supplied by the courtesy of Dr. R. Ting. This line was established by infecting a normal rat kidney line with Moloney sarcoma virus (Gross pseudotype). The cell line was designated NR (MSV-39) and released competent sarcoma as well as leukemia virus.

Immunization of B_6AK mice was done with 2 doses of 5 x 10^6 cells/mouse 2 weeks and 4 weeks apart. Groups of mice were also immunized with a cell-free supernatant of the growth medium. Two weeks after the second immunizing dose the mice were challenged with four different doses of thymic cells (10^6, 10^5, 10^4, 10^3) from leukemic AKR mice. Since the challenge dose was not sufficiently strong to cause death, a second challenge of transplantable AKR lymphomas was administered 23 days after the first challenge. Transplantation resistance was conferred both with cells and supernatant against the 10^4 challenge level; 45% and 32% survived respectively at day 154 against 12% of the non-immunized controls.

Repetition of the above study with X-ray inactivated cells or virus freed from cells by freezing and thawing failed to improve the survival rate.

II. Studies With XM-1

Law and Ting have reported the isolation of an hemangiosarcoma induced by MSV (MLV). The tumor in(C57Bl/Ka x C_3H/Lw)F_1 mice was carried in immunologically deficient mice for several passages. In vivo and in vitro tests for either Moloney sarcoma or leukemia virus were negative. Transplantation

resistance studies demonstrated that this tumor was both immunogenic and immunosensitive. This tumor was of interest to us for two reasons. (1) Since this was a non-releaser tumor, it was an excellent candidate for use as an immunogen in our immunotherapy studies. (2) Preliminary data also suggested that this tumor did not possess transplantation antigens cross-reactive with those induced by Rauscher virus. This represented a departure from a vast body of data collected in several laboratories including our own and we were anxious to confirm this finding.

Primary tumors were induced by Moloney, Friend, and Rauscher virus by inoculation of virus into newborn (C57Bl/Ka x $C_3H/Lw)F_1$ mice. A number of these primary tumors were established in transplant and their transplantation characteristics established. In a series of separate experiments, the antigenic relationship of XM-1 to those antigens induced by FMR type viruses was determined. In each experiment a different FMR virus was inoculated into weanling (C57Bl/Ka x $C3H/Lw)F_1$ mice. One month later infected and control mice were challenged with titrated levels of XM-1, and a syngeneic leukemia induced by the virus used as the immunogen. The results of these studies clearly indicate that XM-1 is at best only weakly immunosensitive. Strong transplantation was obtained in each experiment with the leukemia induced by the virus used as immunogen.

In contrast, no transplantation resistance was obtained with XM-1 in animals infected with Rauscher virus or Friend virus and only marginal resistance (3/9 tumors in immunized mice vs 7/10 tumors vs control mice) was obtained in mice immunized with Moloney virus. In a single experiment no resistance was obtained in animals immunized once with Moloney sarcoma virus. Thus in terms of immunosensitivity XM-1 is only weakly reactive and then only in mice immunized with the leukemia virus represented in the pseudotype sarcoma virus used for its induction.

In view of the weak resistance obtained with XM-1 attempts were made to determine if the tumor retained its immunogenicity. Tumor brei was inoculated into the foot pads of young (C57Bl/Ka x $C_3H/Lw)F_1$ mice. Tumors were allowed to develop in the foot pad for several weeks and then the tumor bearing leg of each mouse was amputated at the second joint. Two weeks later, these mice and sham operated controls were challenged with titrated levels of XM-1. Although the numbers of mice were small, there was a delay in latent period and fewer tumors in the immunized mice vs controls. More animals will have to be included in this study before a final judgment can be made, however, the data are suggestive of immunogenecity for XM-1.

Studies have also been done in this laboratory to confirm that XM-1 is a non-releaser tumor. Animals bearing XM-1 tumor for from 9-44 days were bled and the sera tested for the presence of neutralizing antibody or leukemic and sarcoma virus. Neither antibody nor infectious virus isolated, thus confirming the previous report on the non-releaser status of this tumor.

III. Nonspecific Active Immunotherapy

BCG is an anti-tuberculin vaccine that has been shown also to act to non-specifically increase the host's immunologic system. Preliminary animal

and clinical studies have suggested that through this mechanism it may exert anti-tumor activity. To test this further we first chose an allogeneic system. The DBA/2 tumor, L1210, was transplanted into the H-2 compatible BALB/c mice, and BCG was given. No protection was demonstrated when both tumor and BCG were given on day 1.

A follow-up experiment was done using the LSTRA tumor (a Moloney Leukemia Virus-induced tumor) in BALB/c mice. Five different cell doses were given as the tumor challenge (10^5, 10^4, 10^3, 10^2, 10^1) per mouse. BCG was given 1 day after the tumor challenge, with groups getting either 0.55 cc or 0.10 cc per mouse. Mice receiving 10^5 tumor cells also received cytoxan on day 7. Protection was only observed at the 10^2 tumor cell challenge, and then only with 0.10 cc BCG and not 0.50 cc of BCG. The control animals given 10^2 cell/mouse had an MST of 15 days with 9/10 animals dying on day 17, and 1/10 living for 30 days. The group given 0/10 cc of BCG had no MST with 5/10 living and tumor-free at day 30.

IV. Studies with Asparaginase (with Cooney)

In previous studies we had shown that only a certain portion of C_3H and BALB/c mice treated with L-asparaginase developed HA antibodies. The P1798 tumor was resistant to therapy in the antibody producing mice and fully sensitive in the non-responders and in normal mice. The results of asparaginase clearance from plasma were consistent with the resistance of the tumor in the immune mice. The t 1/2 in the immune mice was <10 minutes and in the normal mice was 75-130 minutes.

During the past year these studies have been extended in several ways. In vitro neutralization studies with high titered anti-asparaginase mouse and rabbit serum indicated that only approximately 30-50% of the activity of asparaginase could be removed. This observation coupled with the rapid clearance of L-asparaginase from the blood of immune mice led us to studies on the L-asparagine concentration in immune and normal animals following the administration of various concentrations of L-asparaginase. We were attempting to determine whether differential rates of L-asparagine depletion could account for the therapeutic difference observed in immune and normal mice.

Following the injection of 0.1 IU L-asparaginase intravenously, the L-asparagine concentration in a normal mouse dropped to 2/3 normal values and remained there for eight hours. In the immune mouse given 0.1 IU no effect on the L-asparagine concentration was noted. With 1.0 IU a ten-fold drop in plasmatic L-asparagine was observed within 1/2 hour, with recovery to normal values within 8 hours. In the immune mouse that received 1.0 IU the L-asparagine concentration fell to 3/4 normal value at 1/2 hr. and was normal at four hours. In normal animals injected with 10 IU, L-asparagine was undetectable at any assay period up to and including twenty-four hours. L-asparagine concentration in immune mice given 10 IU was undetectable at 1/2 hr. with restitution beginning at 2 hours and being essentially complete by 12 hours.

Comparison of these data with chemotherapy trials in normal and immune mice suggest that L-asparagine concentration in the plasma of mice must be

significantly depleted for more than 8 hours to obtain a therapeutic effect. Also, that since some depletion occurred in immune mice intensive treatment with high levels of L-asparaginase might render the tumor sensitive in these mice. More studies involving more animals are required to define these relationships more precisely.

Some chemotherapy studies were done with Erwinia asparaginase in animal resistant to E.coli asparaginase. The results suggested that the Erwinia preparation might be therapeutically efficacious in situations where antibody to E.coli asparaginase had developed.

V. In Vitro Testing of Antiviral Compounds

The recent finding of RNA dependent DNA polymerase associated with RNA tumor viruses and the observation that the enzyme can be inhibited in cell free systems by derivatives of Rifampicin have led to studies with these compounds in virus injected cells. To date, two rifampicin derivatives have been tested.

Rifampicin (NSC-113,926), N-demethyl-rifampicin, (NSC-143,416) and Benzyl-rifampicin (NSC-143,481), were tested for their ability to inhibit Moloney sarcoma and leukemia viruses in tissue culture.

Candidate compounds are tested in two stages:

A. Toxicity to normal mouse embryo fibroblasts is determined on 24-hour old tissue culture. Toxicity is defined as (a) greater than 25% inhibition of the control culture's monolayer and (b) gross changes in the cell morphology.

B. Non-toxic drug level(s) are tested against: (1) Moloney sarcoma virus and (2) Moloney leukemic virus, employing the following specific methods:

(a) Assay against Moloney sarcoma virus. The virus titer is determined at days 4-7. Samples are taken at intervals to assay the virus titer in the supernate.

(b) Assay against Moloney leukemia virus. The titers are determined after 6 days. Supernate samples are collected after 4 and 6 days of incubation and titrated for viral content.

Rifampicin by day 4, 50 mcg/ml completely inhibited the foci formation. However, this concentration also inhibited the normal tissue monolayer approximately 40%, which is considered mild-moderate toxic. A lower dose 25 mcg/ml, caused a 2-fold decrease in viral count at day 4. 12.5 mcg/ml was ineffective. At 7 days no inhibition was observed. In a 48 hr. supernatant taken from the 50 mcg/ml sample at day 5, a 2-6-fold inhibition was measured. A 2-fold decrease occurred in a similar supernatant from the 12.5 mcg/ml treated culture.

N-demethyl-rafampicin. At day 4, 50 mcg/ml caused inhibition by a factor of 1.5; 25 mcg inhibited by a 0.5 factor; and 12.5 mg/ml was without an inhibitory effect. At 7 days, no inhibition was observed. In 48 hrs.

supernatants taken from the 50 mcg/ml and 10.5 mcg/ml samples at day 5, a 2- fold decrease was measured.

Rifampicin and N-demethyl-rifampicin had no inhibitory effect on Moloney leukemia virus.

Benzyl-rifampicin was tested at 5 dosage levels against 2 virus concentrations 1:40,000 dilution containing 70 FFU/plate and 1:100,000 dilution containing 48 FFU/ml. After four days of incubation, 10 mcg/ml inhibited the higher virus concentration 3-fold and lower concentration 6-fold. Five mcg/ml inhibited both concentrations by a factor of 2. Two and one-half and 1.25 mcg/ml had a 2-fold inhibitory effect against the lower virus dilution but had no effect against the high dose. 0.75 mcg/ml was inhibitory by a factor of less than 1. This drug not only reduced the number of foci, but also reduced the size of the foci indicative of suppression of virus production. The great majority of treated foci were approximately 10% of the control size at 10 mcg/ml. None of the concentrations tested were toxic to the cells. Supernated titration 120-144 hour samples showed that 10 mcg/ml inhibited the virus titer by at least 2 logs. Lower drug concentrations (5 mcg - 0.75 mcg) was inhibitory 6-10 fold.

Antiviral Testing In Vivo:

VI. Tilorone

Krueger and Mayer reported that Tilorone hydrochloride (NSC-143696) given orally to mice before they are infected, is active against several RNA and DNA viruses. The compound is effective when given prophlactically; the optimum time of treatment depends on the route of infection. An antiviral serum component is found in mice treated orally with Tilorone. The active material fulfills sufficient biological criteria to be classified as an interferon.

A. Tumor induction with Moloney sarcoma virus in Tilorone treated mice. Male BALB/c mice of 8 weeks were inoculated with $\sim 10^5$ FFU or $\sim 10^3$ FFU of MSV in 0.1 ml IM route. Tilorone treated on 3 dosage levels was initiated 24 hours later with both virus concentrations. Injection was repeated daily until the tumor in the control started to regress. Controls received saline as placebo. No difference in percent tumor takes and in average tumor size exists in groups which received $\sim 10^5$ FFU MSV. In groups which received $\sim 10^3$ FFU, slight decrease in percent tumor takes but more pronounced suppression in tumor size was noticeable.

B. Therapy of AKR spontaneous leukemia with Tilorone.
In leukemic mice, 50 and 40 mg/kg tilorone treatment (every day for 20 days) did not increase the Med S. T. nor the range and percent of survivals. Tilorone treatment for remission maintenance (after 2 doses of Vincristine 0.25 and Prednisone 30 mg/kg) from day 7 was virtually without effect - 13% ILS with 32 mg/kg drug.

The absence of antileukemic activity in AKR mice but the slight to moderately effectivity in BALB/c MSV tumor systems appear to reaffirm Krueger and Mayer's

observations whereas the Tilorone has activity only before virus infection. Pretreatment of BALB/c mice before injection of MSV might yield better results.

VII. BALB/c Leukemia

The Moloney Virus-induced leukemia in BALB/c mice has been treated with 21 different compounds in 68 different dosages. The system differs from the AKR system in that virus is inoculated into newborn mice, the tumor arises at approximately 10-14 weeks of age and a palpably enlarged spleen, but not lymphadenopathy is the method of diagnosis. The WBC count in this system as in the AKR is also not of prognostic significance as is evidenced by a cumulative mortality plot.

The response to chemotherapeutic agents is similar to the AKR system in regard to which drugs are active, but response is for the most part quantitatively less than AKR. From Table VII-I, it is evident that only 7 drugs gave greater than 50% ILS and only three drugs had 100% or greater ILS. Cytoxan at 100 mg/kg, weekly, to death raised ILS 173%. Cytoxan was also tested with an interrupted dose schedule, 141% ILS was achieved with 100 mg/kg, weekly for three weeks. The anti-metabolite, 5-FU is as active in the BALB system as it was in AKR, with a 45 mg/kg dose given Q4D raising the MST 173%. Here again as in the AKR system, a low toxic to therapeutic ratio exists. 5-FU was tested at doses of 31, 45, 64, and 78 mg/kg, with activity confined to the 45 mg/kg dose. The mechanism of action of Cytoxan and 5-FU are known to involve interference in cellular growth and metabolism. However, whether antiviral activity is also present is under study. Cytoxan has already been observed to have no inhibitory effect on the viral titer during the period when antitumor activity is most evident clinically.

For the most part the other drugs tested have demonstrated only mild-moderate activity. Of interest are two new agents, Isophosphamide and Trilophosphamide, two structurally similar compounds to Cytoxan, which have given 115% and 80% ILS, respectively. The new drug ICRF-159, while inactive in AKR mice, caused a 66% ILS in BALB/c mice at 200 mg/kg Q4D.

Combination therapy has also been studied in the BALB/c system. The most effective combination has been CTX 100 mg/kg, weekly, and Ara-C 22.5 mg/kg, Q4D raising the MST 294%.

Cytoxan was also given with BCNU in multiple dosage combinations. Maximum effect occurred when Cytoxan was given at less than its optimal dosage (50 mg/kg). Best combination was Cytoxan (50 mg/kg) + BCNU 10 mg/kg. Both given weekly from day 1, raising the MST 109%. Vincristine-prednisone combinations have been consistently less effective than in the AKR tumor with only a 39% remission induction success rate. Sixty and 90-day survivors were found mostly in groups treated with Cytoxan. Particularly effective was the combination of Cytoxan and Ara-C which had 7/19 mice living 60 days and 4/9 living 90 days.

VIII. AKR Leukemia

During the past two years the spontaneous AKR lymphoma-leukemia system has been tested with 32 different drugs employing 123 dosage schedules. Therapy protocols have included single drug trials, combination trials and two-stage experiments utilizing vincristine and prednisone in combination to induce remission followed by various drugs attempting to maintain remission.

The establishment of a reliable diagnosis is important in order to obtain a repeatable median survival time for untreated controls. To confirm last years report that the WBC count has little diagnostic or prognostic value a retrospective analysis of 243 untreated leukemic mice was done. Forty percent of these mice have a WBC greater than 20,000 (average 30,000) and an 11-day MST. Sixty percent with less than 20,000 WBC (average 11,000) also had an 11-day MST.

The relationship between response to treatment and WBC count was studied in therapy protocols using cytoxan and Ara-C, singly and in combination. No significant trends were evident in groups separated by WBC count and then treated with the same drug. With cytoxan therapy the high WBC count group had 238% ILS and the low count -- 187% ILS. Using cytoxan and Ara-C in combination the low WBC count group had 187% ILS compared to 123% in the high count group. Based on these studies, the diagnosis continues to be done by palpation of enlarged spleen and lymph nodes, without regard to the WBC.

Of the 32 compounds tested, six had 90% or greater increase in MST, which was significant using a modified T test at 99 or 95% confidence level (Table VIII-I).

Cytoxan has consistently been one of the most effective therapeutic agents in this system with an ILS varying between 55-377% depending on the dose employed. Early studies involved treating animals weekly to death; However, because of the possibility that this was killing animals with toxicity we have run interrupted dosage schedules. The optimal schedule appears to be cytoxan at 100 mg/kg weekly for 5 doses, giving 177% ILS.

5-FU has been almost as active as cytoxan in the AKR system. A low toxic: therapeutic ratio is evident with an optimal dose appearing to be between 45-64 mg/kg, with ILS of 129% and 274% increase, respectively. Interrupted doses were employed here also, with less consistent results. The other 5 active agents in this system do not appear to represent any single class of compounds. 5-Azacytidine (NSC-102816), raised the MST 160% at 12 mg/kg, Q4D. The 2 cytoxan analogues, Isophosphamide (NSC-109724) and Trilophosphamide (NSC-109723), were moderately effective with an ILS of 107% and 56% respectively at their optimal doses. The nitrosoureas also demonstrated some activity ranging from a 90% ILS for Me-CCNU to 40% ILS for CCNU, with BCNU at 75%.

Of particular interest is the schedule dependent agent Ara-C which has been given previously without demonstrable activity. When the depot form, Palmo Ara-C (NSC-135962), was tested using a variety of doses and treatment schedules activity up to 113% ILS was demonstrated at 15 mg/kg, daily for 15 days.

The two clinically active anti-metabolites, 6-MP, and MTX were inactive in the AKR system, for either remission induction or remission maintenance.

Protocols utilizing remission-induction followed by remission maintenance were established to mimic certain clinical situations and to gain the advantage of observing the response of fairly uniform groups to sequential chemotherapy. We defined successful remission induction as the state when spleen and lymph nodes were no longer palpably enlarged. The effectiveness of vincristine-prednisone in combination had been demonstrated by Schabel and coworkers. In vitro bioassay studies with this combination reduced by 4-5 logs the number of leukemic AKR thymic cells. Also, this is still one of the most effective regimens for inducing remission in childhood acute lymphatic leukemia. A summary of five remission inductions with vincristine and prednisone is tabulated in Table VIII-2 following 2 doses of 0.5 or 0.25 mg/kg VCR and 30 mg/kg prednisone on days 1 and 5. Remission induction was successful in 58-71% with an average of 65%. Following successful remission induction mice were randomized into treatment groups designed to prolong remission, and these were defined as remission maintenance groups. In each experiment (except Expt. 2) mice were maintained on placebo and constituted the control groups. MST for this control group was consistently 20-21 days. Using the vincristine-prednisone remission induction,several drugs given as "maintenance therapy" were found to provide a statistically significant increase in the MST (at the 99 or 95% confidence level). Cytoxan at either 150 or 100 mg/kg, weekly, provided the most effective maintenance therapy, 150 and 140% ILS, respectively. 5-Azacytidine was also effective, increasing the MST 64%, at 6 mg/kg every 4th day. Two structurally similar compounds, chloroethyl imidazole carboxamide and dimethyl imidazole carboxamide raised the MST 30 and 40% respectively.

Long-term survivors (60 or 90 days) have been infrequent in the AKR system, occurring for the most part with cytoxan-treated groups, either singly or in combination with other active agents. The questions raised by these long-term survivors and their eventual death with disease deserve further study. Surviving Lymphoma cells may explain reinduction of the disease but another possibility may be reinduction by the persisting leukemia virus.

Although we have established the virus level in the serum of untreated AKR mice, the possible antiviral activity of the drugs tested has not been assessed. However, we know from our study with BALB/c primary leukemia that cytoxan does not affect the virus level despite its anti-leukemic activity.

IX. Immunotherapy Studies

In previous studies it had been demonstrated that specifically sensitized allogeneic cells from various F_1 strains or from DBA/2 mice were capable of eradicating residual Moloney virus induced leukemia in BALB/c mice. It was also shown that although many of the cured mice were chimeras, this was not an absolute requisite for the therapeutic effect. During the past year emphasis has been placed on the following areas.

Studies on the late deaths in cured mice. One of the disturbing aspects of immunotherapy model used in these studies was the many mice apparently cured of their transplantable lymphoma died 60-100 days post implant. Some of the mice obviously died from starvation because of loose teeth but we have been able to establish that the major cause of death is the induction of primary

sarcoma and leukemias in these mice. The immune cell donors were immunized with Moloney sarcoma virus and the spleen cells from such mice harbor either undetectable amounts of competent virus or the MSV genome. We have examined 18-20 moribund long-term survivors about half pathologically and half virologically and in all but two have been able to either identify sarcomatous tissue or isolate in vitro systems murine sarcoma virus. Thus while MSV is an efficient immunogen, it appears to severely limit the possibilities for obtaining normal life spans with the cured animals.

Immunotherapy of primary tumors. In view of the success experienced with the immunotherapy model using transplantable MLV-induced leukemia attempts were made to treat primary leukemias. Four large experiments were performed during the past year with essentially negative results. One of the major problems is that the advanced stage of disease in these mice at the time they are subjected to therapy, limits the amount of drug they will tolerate. Doses of 180 mg/kg cytoxan or greater are required to establish chimerism in BALB/c mice and such drug levels approach the lethal range for BALB/c mice with primary leukemia. In the initial experiments which involved a single dose of cytoxan followed by various normal or sensitized cell populations the above considerations were thought to be the limiting factors. To circumvent these problems, a large experiment was done which involved administering a single dose 150 mg/kg cytoxan to reduce the tumor level and improve the condition of the mice. Animals that survived for two weeks were regrouped and given either 240, 210, or 180 mg/kg cytoxan followed either by immunized, normal or no cells. The median survival times after regrouping for the three drug levels are 34, 33, and 46 days, respectively. In no instance was the survival improved by the administration of either normal or sensitized cells. In view of the success obtained in the transplantable tumor system and the lack of success against primary tumors, it must be considered that re-induction by the virus may be playing a limiting role in obtaining a chemo-immunotherapeutic effect.

Graft vs Host Disease

a. BALB/c - DBA/2 system - The observation that DBA/2 spleen cells from animals immunized with normal BALB/c somatic antigens would provide significant increases in survival time of tumor bearing animals treated with cytoxan led to an extensive series of experiments. These results were erratic in that spleen cells from animals sensitized to normal somatic antigens did not always provoke the same degree of Graft vs host disease (GVH). In those cases where GVH was not limiting, increases in MST of one to two or more weeks greater than those observed with drug alone were obtained. Many of the successfully treated mice died without gross evidence of tumor and appeared to be suffering from chronic GVH. Attempts are being made to eliminate the GVH by destroying the lymphoid graft with specific antisera. To date, some procedures have diminshed the graft but none has entirely eliminated it.

b. BALB/c - C57Bl/6 system - In contrast to the previous system, prompt GVH is invariably produced when H-2 incompatible cells are injected into cytoxan treated BALB/c mice. In pilot experiments it was demonstrated that GVH could be prevented if antisera directed against the graft was administered 2 days after engraftment. When the antisera was withheld for 4 to 6 days no deleterious effect on GVH was observed. Immunotherapy trials were done with

C57Bl/6 cells sensitized to MSV and to normal BALB/c tissue and with unsensitized cells in a situation where the graft was broken with specific antisera 2 days after engraftment. In all instances cytoxan treated animals that received C57Bl/6 cell died of GVH. In those groups in which the graft was destroyed by antisera, the median survival times were similar to that of the animal treated with cytoxan alone. Thus even the specifically sensitized cells, the immunological effect is not obtained within 48 hrs. of engraftment.

In order to determine the residence time required for the graft to effect eradication of residual leukemia, experiments were done with (C57Bl/6 x BALB/c) F_1 cells in BALB/c mice. The results of these experiments suggest that approximately a 4 day graft life is required for a therapeutic effect to be obtained.

Significance:

The purpose of these studies is to obtain a better understanding of the nature and biology of tumor associated antigens, the immunological responses elicited in the tumor bearing host, the effect of chemotherapeutic agents on these responses, and the contribution immune phenomena might make to the overall therapeutic effect. The knowledge derived from such studies can be applied to model systems with transplantable and primary tumors in mice involving immuno-therapy and immuno-chemotherapy, and this may lay the frame work for subsequent clinical studies.

Proposed Course:

During the coming year major effort will be in the following areas: (1) Chemotherapy studies with primary lymphomas and leukemia; (2) Immuno-chemotherapy with primary tumors involving specific and non-specific immunogens; (3) The development and use of model systems for the control of graft vs host disease; (4) Transplantation, serological and virological studies with virus-induced non-producer tumors; (5) In vivo and in vitro studies with RNA dependent DNA polymerase inhibitors; (6) Further studies on the relationship of asparaginase concentration, asparagine depletion and therapeutic response with P1798.

Publications:

See current Organization Report, Office of the Chief, Drug Evaluation Branch.

Table VII-I

Individual Drug Trial of Moloney Viral Induced Leukemia in BALB/c Mice

Percent Increase in Median Survival Time		
100% or greater	50-99%	Less than 50%
Cyclophosphamide	5-Azacytidine	Platinum
Isophosphamide (NSC-109724)	Trilophosphamide (NSC-109723)	Procarbazine
5-Fluorouracil	BCNU	Chromonycin (NSC-58514)
	ICRF-159	Daunomycin
		CCNU
		Cytosine arabinoside
		Prednisone
		Vincristine
		Me CCNU
		Emetine
		Ellipticine
		Camptothecin
		Propanol dimethane sulfonate
		Propanol iminodidimethane sulfonate

Table VIII-I

Individual Drug Trial of Spontaneous AKR Leukemia

Percent Increase in Median Survival Time

90% or Greater[A]	89-50%	Less than 50%
Cyclophosphamide	BCNU	CCNU
5-Fluorouracil	Propanol Dimethyl Sulfonate	Dimethyl Imidazole Carboxamide
5-Azacytidine	Propanol Iminodi-dimethane sulfonate	Daunomycin
Palmo-O-Ara C	Prednisone (1)	Vincristine
Methyl CCNU	Sarcolysin	Procarbazine
Isophosphamide (NSC-109724)	Chloroethyl Imidazole Carboxamide	Camptothecin
	Trilophosphamide (NSC-109723)	Emetine
		Chromomycin
		Ellipticine
		Platinum
		6-Mercaptopurine
		Methotrexate
		Picolinaldehyde 5-H Pyrazole Thiosemicarbazone
		Actinomycin D
		Aminothiadiazole
		ICRF-159
		Vinblastine
		Prednisone (2)

[A] Significant on 99% or 95% Confidence Level.

Table VIII-2

Remission Induction with Vincristine and Prednisone
In Leukemic AKR Mice

Drug	Dose[A] Mg/Kg	No. of Mice in Test	Percent of Mice in Remission	Med. S. T.[B] (Days)	Range of Death (Days)
VCR Pred	0.5 30	116	58	20	11-48
VCR Pred	0.5 30	94	69	ND[C]	ND[C]
VCR Pred	0.25 30	111	71	21	10-33
VCR Pred	0.25 30	149	64	20	10-80
VCR Pred	0.25 30	156	65	21	9-

[A] On Day 1 and 5
[B] After remission induction 13-16 mice were injected with saline every 4 days until their death.
[C] ND - Not Determined

Screening Section

Drug Evaluation Branch

Objectives and Significance to the National Cancer Institute

Principal objectives of the Screening Section are the selection and recommendation of new drugs as candidates for development toward clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known antitumor drugs. Objectives are attained by (a) screening a large number of synthetic and natural products in vivo, and to a limited degree, in vitro; (b) conduct of biological assays for directing fractionation of natural products and isolation of active components; (c) in-depth evaluation of materials which emerge from primary screens with emphasis on the influence of factors such as route and treatment schedule that affect therapeutic efficacy; (d) analysis, evaluation, and presentation of pertinent experimental results to Chemotherapy Staff; and (e) methodology research leading to development of improved assays for drug evaluation. Program is implemented through establishment and coordination of work conducted by contractual arrangements with qualified institutions and through intramural laboratory studies. Activity against experimental tumors in vivo is the principal basis for recommending candidate drugs for development toward clinical trial and for studying optimal conditions for drug usage.

In vitro activity against mammalian tumor cells or microorganisms provides a means of recognizing biological activity among synthetic agents submitted in insufficient quantity for in vivo screening, for following fractions and isolation of active components of crude natural products with correlative in vivo activity, for providing information relative to mechanisms of drug action, and for developing assays for estimating effective drug levels in blood and tissues.

I. Staff Functions

A. General

The Screening Section is staffed by six senior professional and two junior professional personnel, one on detail from Office of the Chief, DEB, one statistical clerk, one technician and one secretary. Principal investigators are: B. J. Abbott (Head); D. Abraham (CO); M. C. Baratta (CO); R. I. Geran; N. H. Greenberg; and M. M. MacDonald. In addition, staff is supported by six technical and statistical part-time aids employed under work-study plans. The latter are employed primarily in staff supervised research projects in the section's intramural laboratories. Major findings of the intramural laboratory program are summarized in current individual Project Report, Serial No. NCI-3578.

The present administrative report is essentially devoted to the contract program.

As of March 1, 1971, staff served as Project Officers for three contracts devoted predominantly to in vivo screening; Hazleton Laboratories (PH43-63-622), Illinois Institute of Technology (PH43-68-1021), and WARF Institute (PH43-66-494) and two contracts for in vitro screening methodology; University of Miami (NIH 70-2225) and University of Wisconsin (PH43-67-1126). At the time of this writing, two additional contracts for in vivo screening are being initiated, following competition of all in vivo and in vitro service contracts. In addition, staff, in collaboration with the Chief, DEB; Chief, DDB; Associate Scientific Director and Chief, CCNSC; and Scientific Director for Chemotherapy, monitored in vivo and in vitro screening, secondary drug evaluation, and methodology tasks performed under multifaceted contracts with Southern Research Institute (NIH 71-2098), Arthur D. Little, Inc. (PH43-65-61), Bristol Laboratories (PH43-69-35), and Microbiological Associates, Inc. (PH43-68-1283). In reviewing biological effects of natural product fractions the Screening Section established directions of effort under additional CCNSC contracts for natural product fractionation and isolation. In contract related activities, staff monitor the work of contract laboratories as indicated; coordinate total effort with other CCNSC and Chemotherapy projects; design assays for selection of new active drugs, investigate usefulness and feasibility of new assays; recommend candidate drugs for clinical trial; and prepare data for publication and for review by the Drug Evaluation Committee, Operating Committee, Experimental Therapeutics Area, Clinical Trials Area, and for Chemotherapy Decision Network review in accordance with the program's task convergence format.

B. Specific

In conduct of duties staff (1) review proposals for institution or renewal of contracts, recommend new project to Chief, DEB and prepare materials for Program and contract reviewing bodies; (2) provide guidance and instruction to contract laboratories, monitor scientific and technical sufficiency, and indicate areas needing modification or clarification through frequent communication and project site visits; (3) establish priorities for testing and instruct laboratories to conduct non-routine experiments of special interest; (4) design experimental protocols for screening and detailed drug evaluation, define parameters of response, establish criteria for "activity"; (5) advise Program Analysis Branch (PRA), CCNSC, of data retrieval needs and collaborate in establishment of procedures; (6) advise Mammalian Genetics and Animal Production Section, CCNSC, of animal requirements; (7) organize, review, and summarize experimental results; (8) instruct laboratories regarding needed additional data; (9) advise natural product chemists under contract to Drug Development Branch of crude materials worthy of fractionation and isolation; (10) conduct studies related to the

biological quality control of experimental clinical formulations, (11) conduct studies leading to solution of drug evaluation related problems arising during toxicological study or early clinical trial of new drugs; (12) design and conduct basic research leading to the development of improved assays for screening.

Senior staff serve on the Drug Evaluation Committee (DEC), chaired by the Chief, DEB, which recommends to Chemotherapy Staff materials which meet criteria for acceptance as clinical candidates. Four of the staff constitute the DEC's Data Review Subcommittee (DRS) which reviews screening results, determines adequacy of testing, acquires additionally required data, and submits to full committee materials meeting minimal criteria for significant and reproducible activity. Staff collectively form the Screening Committee (chaired by the Section Head) which assigns materials that pass Decision Network 2 (DN-2) to contract laboratories for initial route and schedule dependency studies and for additional testing against a limited spectrum of signal rodent tumors; and summarizes data for consideration at the Decision Network 2B and subsequent stages of the linear array. Thus, the Screening Section Staff represent a major part of an integrated program for the evaluation of more useful antitumor chemotherapy and the intensive study of new agents.

II. Significant Accomplishments

Accomplishments are summarized below in accordance with performance of specific tasks related to (A) determination of antitumor activity of new agents, (B) selection of candidate drugs for development toward clinical trial (C) detailed evaluation of new and old drugs of clinical interest, and (D) methodology, research and development.

A. Determination of Antitumor Activity of New Agents (Primary Screening)

Primary screening consists of the original testing of synthetics, plant, fermentation, and animal products submitted to the CCNSC. Protocols for screening are designed to select initially a modest number of materials which are then subjected to progressively exacting requirements designed to elicit those with the greatest antitumor potential. During calendar year 1970, 8,409 new synthetics, 3,987 new crude plant extracts, 2,172 new crude fermentation products, and 587 new crude animal products were submitted to the screening program. Table 1 shows the number of computer processible screening tests conducted for various types of materials in each assay system. Until August, 1970, all materials were routinely screened *in vivo* against L1210 utilizing two treatment schedules (single and daily treatment) to the extent permitted by the supply of material. After the analysis of data for one hundred compounds subjected to schedule dependency studies, it appeared that an every fourth day schedule (with treatment on

days 1,5, and 9) might increase the yield of active materials in L1210, and be a more advantageous predictor of toxicity than the single treatment. Based upon this study, the daily treatment schedule was retained for L1210 screening of all materials in sufficient amount, and the q4D schedule was added to the L1210 protocol and given priority over single treatment when the amount of submitted agent is insufficient for the conduct of the three schedules. The L1210 screen has continued to uncover a modest number of active materials from among "synthetic" materials and to be highly resistant to natural products. For this reason, use of the P388 lymphocytic leukemia as a natural product screen to uncover materials which might display correlative activity against L1210 upon purification has continued. Discussion of current analyses regarding the usefulness of this approach appears in the report of the Chief, DEB. Efforts were intensified during this report period to find correlative in vivo systems for crude natural products under fractionation in the Walker 256 - IM(5WM) rat tumor assay. It is currently anticipated that almost all natural product testing in the 5WM will be terminated by July 1, 1971. Materials under development toward clinical trial and their analogs continue to be evaluated in the W256-IP (survival) assay. Although severely hampered by the reduced test capacity in the total screening program, limited screening was conducted against the B16 mouse melanoma in order to aid in evaluating this assay. The Lewis Lung mouse carcinoma was used to a limited degree as an ancillary screen and, more extensively in the secondary evaluation of compounds in development toward or in clinical trial. Other assays were used in connection with special studies including activity against spontaneous and first generation transplantable AKR leukemia, drug resistant tumor variants, evaluation against a tumor spectrum of drugs reported as active from other programs but which were inactive in CCNSC routine primary screens. In vitro (KB) screening continued against all new crude plant and animal products and fractionation products from crude plant extracts which had demonstrated correlative in vivo activity. Of the new synthetic agents submitted to CCNSC, 1.4% were received in insufficient quantities for in vivo testing under our revised protocols, and these were screened in vitro.

B. Selection of Candidate Drugs Toward Clinical Trial

During calendar year 1970, 135 new materials passed the initial stages of screening and 238 materials were reviewed by DRS and/or DEC (Table 2). Six of these 238 drugs were judged by the DEC to have met current criteria for acceptance as clinical candidates and were referred to Chemotherapy Staff for DN-2 consideration. Seven drugs including five of these six passed DN-2. Table 3 lists the materials which passed DN-2 in calendar year 1970 and the experimental basis for the selection of each. Examination of the basis for selection of clinical candidates during this and previous years emphasizes that the L1210 is a relatively

insensitive tumor. While the positive correlation between anti-L1210 activity and clinical efficacy indicates that it should continue as our principal primary screen, its use to the exclusion of other assays has resulted in a decrease in the total number of new active agents available for development. Institution of a second tumor system as screening assay is of importance. Preferably, such an assay should be different from those which have been widely used and terminated and should be logically based on experimental evidence that it might predict drugs of usefulness against slow growing tumors. At present the Lewis Lung carcinoma and/or B16 melanoma appear to be prime candidates (see Section D, this report).

C. Detailed Evaluation of New and Old Drugs of Clinical Interest

The Screening Section is largely responsible for the detailed evaluation in rodent tumor systems of drugs which passed DN-2 including route and schedule dependency tests, combination chemotherapy, studies of comparative effects against fast and slow-growing tumors, and other appropriate studies to determine the extent to which modifications in drug usage might increase therapeutic efficacy leading to cures of experimental tumors. The section reviews and summarizes such information for consideration of Chemotherapy Staff at Decision Points subsequent to initial acceptance. Comprehensive records of experimental results are maintained by the section of the diverse assays, for active drugs and other agents of special interest: the Selected Agents (SAC) File. The restructuring of this file in accordance with the Chemotherapy Program's Linear Array Format has been accomplished.

1. Route and Schedule Dependency Studies

During calendar year 1970, 39 compounds were subjected to a total of 53 route and schedule dependency studies in accordance with the current protocol. Thirty-one studies were conducted against L1210, 11 against P388, four against B16 melanoma, one against Lewis Lung carcinoma, and six against W256. The Screening Committee reviewed and submitted to the Chief, DEB, summarized reports of such studies for 40 compounds, 35 of which were considered to have been adequately tested, and requested additional follow-up work for five. Initial route and schedule testing is designed to determine the effect on therapeutic effectiveness when the interval between treatment is varied. The usual Type I route and schedule dependency test involves IP treatment on Day 1 only, QD for five days, QD for nine days, every fourth day X 3, every eighth day X 2, every three hours on Day 1 only, every three hours q4D, and every three hours q8D. In addition, attention is given to the determination of activity via the SQ route as indication of the effectiveness of parenteral treatment at a site different from that of tumor implantation, and via the oral route

as an indication of absorption from GI tract. The IV route is included when feasible. In general route and schedule dependency tests are conducted using parallel groups of tumor-bearing and normal animals as an aid to estimating the degree to which lethal toxicity influences schedule dependency. It should be noted that the number of schedule dependency tests conducted exceeds what one might expect from the number of materials which passed DN-2. Usually for each drug, studies subsequent to the initial Type I test are required for confirmation of schedule dependency, determination of schedule dependency via various routes, and determination of schedule dependency of clinical formulations. In addition, the selection of the clinical candidates from active members of a structural class has required comparative schedule dependency testing. Compounds with very extensive activity against the initially observed sensitive tumor may not appear to be schedule dependent because of the extreme sensitivity of the tumor. In such cases, schedule dependency tests are conducted against more advanced forms of the tumor and against other tumors with less inherent sensitivity to the compound.

2. Combination Chemotherapy

Based upon the emergence in recent years of a substantial data base pointing to the clinical effectiveness of drug combinations, there has been increased emphasis on the conduct of therapeutic trials in animals of new drug combinations which might lead to the development of more effective and curative therapy in the clinical situation. While the CCNSC has been engaged in combination chemotherapy for a number of years through its contract laboratories and since much of the design for combination therapy is developed within these laboratories, the lack of programs for the automatic processing of results has hampered staff monitoring of these investigations. In the past year, working in cooperation with the PRA, an automatic data processing system has been developed for reporting of these results. Data forms have been developed and are in routine use for reporting of this information and the program has been tested on a trial basis. It is anticipated that within the coming year processing of this data will become routine. The bulk of combination studies conducted during the past year have been carried out in our intramural laboratory (see Serial No. NCI-3578), at Microbiological Associates, Inc. (67-1126) as reported in Individual Project Report, Serial No. NCI-3576, and at the Southern Research Institute (71-2098). These laboratories have, through a collaborative effort, made a concerted effort to initiate evaluation of all compounds in development toward clinical trial in limited combination studies. Related major findings are summarized in the reports cited above, in the Annual Progress Reports of the contract laboratories involved, and in the current report of the Chief, DEB.

3. Biological Quality Control of Experimental Clinical Formulation

As a support function, we are collaborating with the DDB, Clinical Drug Distribution Section, in comparing the in vivo activity of formulated drugs with the activity of the bulk compound. The screening system which signaled the activity of the bulk drug is used for this comparison. In addition, considerable effort has been expended in studying the toxicities of non-routine vehicles anticipated for clinical formulation. Such studies can be extensive inasmuch as the potential toxicity of a vehicle must be examined carefully, especially when intensive parenteral treatment schedules are indicated. In the past year, comparisons of experimental and final formulations with bulk material have been carried out for 12 drugs in development toward clinical trial and were reported to the Drug Distribution Section.

4. Further Evaluation of "Deferred" Compounds

Among those compounds maintained in the SAC file are a group which have demonstrated reproducible activity in a number of tumor systems, but lack sufficient activity to be considered as clinical candidates. Since many of these represent new classes of compounds, it is felt that they merit further study. For this reason, we are in the process of re-examining these "deferred" materials. In many cases, minimum but significant activities were observed using obsolete screening protocols. To the extent permitted by the amount of material available, approximately 400 compounds have been sent for L1210 testing using the Single, Daily and Intermittent treatment schedules. As the new data is received, it will be reviewed in accordance with current criteria and practice.

5. Other Studies

Additional studies of materials which are in early phase 1 clinical trial or regarded as the most likely candidates for early clinical trial included investigations related to the (a) influence of the tumor site on drug effectiveness (e.g., effects against intracranially inoculated tumors as an indication of the hindrance to therapy of the "blood-brain-barrier"), (b) effect of new therapy on the development of resistance or on overcoming resistance to treatment, and (c) mechanisms of drug action.

D. Methodology (Research and Development)

Research and development consists primarily of studies to establish experimental designs which may lead to improved methods of drug evaluation and to the selection of drugs with greater clinical

potential. A major portion of this work is devoted to the establishment of experimental protocols for screening and the evaluation of additional test systems and their correlations with both the current test systems and with clinical effectiveness. The official CCNSC protocols for screening were last published in 1962, and many modifications have been made in the ensuing years. While such changes in protocols are transmitted on a regular basis to those closely associated with the program, they are not readily available to the scientific community in general. A new edition of the protocols for testing is being prepared for publication. An important factor in formulating protocols for testing to insure reproducibility among a number of laboratories is the selection of appropriate positive control agents. A successful search was undertaken to select a single compound that could be used as the positive control for both the L1210 and P388 screens. Such a compound should be available in large quantities, "shelf-stable", water soluble, stable in solution, and reproducibly active in the appropriate test systems. In addition, its optimal dose should be reasonably high and its dose-response curve sufficiently flat to minimize the effect of temporal and biological variation on reproducibility of activity. This project developed from the Drug Development Branch's request for the recommendation of an alternative to NSC-60339 which had been used in P388 testing, inasmuch as the CCNSC supply was becoming exhausted and the agent is moderately expensive and difficult to synthesize. Me-GAG (NSC-32946) was considered but after comparative testing in nine laboratories, the degree of conformance of results from test-to-test and among labs was considered insufficient for its use as a P388 positive control. Existing data for approximately 50 agents were reviewed with respect to their stability, availability and cost, spectrum of antitumor activity, clinical status, and status in our drug developmental program. Five agents were selected for consideration on the basis of P388 activity. About this same time, the intermittent treatment schedule was being considered as an addition to the L1210 screen. In terms of cost efficiency the selection of a single positive control compound for four test systems; daily treatment of P388, and single, intermittent, and daily treatment of L1210 was considered desirable. Thus, the five candidates for the P388 positive control and approximately 20 L1210 actives were reviewed and retested when necessary for their applicability in all four systems. After reviewing these results and consultation with the Drug Development Branch, NSC-19893 (5-FU) was selected as the current positive control for both P388 and L1210 screening. The decision was based on the reproducibility of its antitumor effects under the different experimental protocols and on the previously discussed factors. It is somewhat less than ideal because of its limited solubility at the high doses used in single treatment. However, it was judged to be the best candidate considering overall requirements. While this report on the search for a new positive control compound might seem unduly detailed, the Screening Section felt

that the importance of this aspect of its program should be emphasized and that the considerations leading to the selection of a positive control agent should be highlighted because of the substantial amount of testing involved (779 tests).

Efforts are continuing to be directed to the study of experimental tumors with relatively long doubling times. The search for a suitable model for screening, as a part of the continuing effort to uncover drugs of clinical usefulness against solid tumors, has been hampered in the past year by the limited screening capacity available in the total program. However, attention has been given to the testing of a number of known active and inactive materials against the IM Lewis Lung (LL) tumor using both tumor diameter and survival time as parameters of response. Compounds under study have included selected synthetics and natural product materials previously found active in the old LL-tumor weight inhibition system. Schedule dependency studies have been carried out against early and advanced LL with drugs of special interest such as Me-CCNU (NSC-95441) which was outstandingly active against established LL and accepted as a clinical candidate on this basis. Bleomycin (NSC-125066) which was accepted for clinical trial in our program on the basis of Japanese reports of experimental and clinical efficacy, was found active against LL and is currently being studied for route and schedule dependency in this system.

Studies against B16 were limited to known compounds of clinical interest. A small number of agents were examined for schedule dependency against B16. It was noteworthy that SC-inoculated B16 was less sensitive than the IP-inoculated tumor to IP treatment. The selection of a "slow growing" tumor as an adjunct to the basic L1210 screen will require repeat testing of a number of materials and the screening of a reasonable number of random acquisitions in order to establish a data base sufficient for analysis of responses and for establishing quantitative criteria for activity. In addition, selection will take into account the kinetics associated with tumor cell proliferation and extent of metastasis. The relative effectiveness of a number of drugs of clinical interest against L1210, P388, B16, and LL is shown in Table 4. Information related to tumor cell growth kinetics for a number of tumors under current development in comparison with L1210 is presented in Table 5.

III. Related Projects

There have been many changes in the protocols for screening since their last publication in 1962. While these changes over the years have been transmitted to those associated with the CCNSC program, the need to publish a comprehensive edition has been recognized for some time so that these procedures would be available to the scientific community in general. A draft of these protocols reflecting the many modifications over the years and our most current instructions is now ready for distribution to staff and screening contractors

for review. Protocols and assay systems will continue to change, but publication in near future of these revised protocols is expected to give us again a complete, fundamental article which will be sufficiently comprehensive to need a minimum number of revisions.

In order for the screening program to function properly, all contract laboratories must employ both tumor lines with uniform biological characteristics and host animals of uniform high quality. To achieve this uniformity, the Screening Section maintains frozen tumor banks at specified laboratories and from these, new tumor lines are dispensed to all screening laboratories at specified intervals. In addition, the tumor lines are monitored by the Screening Section and if it appears that the characteristics of a tumor line in use may be changing, new tumor lines are requested and sent to all contract laboratories from the tumor bank.

The need for host animals in good health and in large numbers necessitates close liaison with the Mammalian Genetics and Animal Production Section. Selected screening contract laboratories perform studies related to the diagnosis and control of animal disease and to provide the quality control necessary for maintenance of the quality of the animals utilized in the CCNSC program.

The Screening Section, as the major user of the CCNSC data processing system, continues to work closely with PRA by advising and assisting in the planning of modifications and the monitoring and review of data necessary to an ongoing data processing system. In addition, we continue to review for need, all requests for special queries to our data files.

IV. Proposed Course of Projects

The Screening Section will continue the supervision of testing and the evaluation of data for synthetic compounds, plant, animal and fermentation products which are submitted to the CCNSC. Emphasis will continue to be placed upon several diverse but related projects of interest to the Chemotherapy Program of the NCI. Utilization of in-house laboratory facilities to provide rapid and accurate evaluation of both test systems and compounds of major program interest will continue. This will involve basic research in areas related to cancer chemotherapy and pilot systems aimed at devising new and improved tumor assays prior to their establishment in contract laboratories. Effort will continue both in the intramural program and in the contract areas to devise, implement, and evaluate more sophisticated systems to uncover drugs of potential clinical usefulness. Development of a broader program for drug evaluation against AKR lymphoma is underway.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

Table 1

Number of Computer Processed Screening Tests Conducted
Calendar Year, 1970

Test System	Synthetics	Plant Products	Fermentation1/ Products	Total
In Vivo				
Leukemia L1210	52,065	10,101	10,350	72,516
Leukemia P388	2,861	25,230	8,375	36,466
B16 Melanoma	1,329		13	1,342
Walker 256				
(Tumor Inhibition)	767	2,176	1,914	4,857
(Survival)	1,169	32		1,201
Lewis Lung				
(Tumor Inhibition)	506	1,235		1,741
(Survival)	255	1,033	4	1,292
Others	474	12	18	504
Toxicity Studies (Non-tumored Animals)	3,559			3,559
Total In Vivo	62,985	39,819	20,674	123,478
Cell Culture	530	7,015	744	8,289

1/ Includes 941 animal products

Table 2

Status of Active Compounds
Calendar Year, 1970

New actives	135
No. of compounds reviewed by DRS and DEC	238
DRS	170[1]
DEC	101
Recommended to Chief, CCNSC as candidates for Chemotherapy Staff (DN-2) consideration as potential candidates for clinical trial	6[2]
Considered (reviewed) by Chemotherapy Staff	9[3]
Passed DN-2	7[4]
Route and schedule dependency studies (experiments) scheduled	53[5]
No. of compounds reviewed by Screening Committee	40[6]

1/ 33 of these were further reviewed by the DEC

2/ B peltatin - January 19, DEC minutes - not included
Included are: NSC-106443, 129942, 101887, 135758, 77587, 78409

3/ These include: NSC-101887, 135758, 114924, 118994, 129185, 77037, 106977, 112907, 136592

4/ These are: NSC-135758, 118994, 77037, 106977, 112907, 129185, 136592

5/ 39 different drugs

6/ 35 listed as complete in test systems reviewed

Table 3

Compounds Passing Decision Network 2 During Calendar Year 1970

NSC	NAME	BASIS	DATE
77037	Tetrandrine (+)	WA256	1/13/70
106977	L-Asparaginase, from Erwinia Carotovora	Replacement for NSC-109229	6/22/70
112907	Hydrouracil, 1-β-D-ribofuranosyl	Adjuvant for NSC-63878	6/22/70
118994	Digylcolaldehyde, α-(hydroxy-methyl)-α'-(6-hydroxy-9H-purin-9-yl)-	L1210	7/20/70
129185	4-Hexenoic acid, 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl- Mycophenolic acid	WA256	3/31/70
135758	Actinomycete fermentation, product from	L1210	7/20/70
136592D	1,4-Naphthoquinone, 2-(3,3-dichloroallyl)-3-hydroxy-, sodium salt	WA256	6/22/70

Table 4

Relative Antitumor Activity of Agents of Clinical Interest
Maximum ILS(%) Against Early Tumor Using IP-Daily Treatment

NSC NO.	Agent	L1210	P388	B16(IP)	B16(SC)	LL
740	MTX	100	122	Neg	Neg	Neg
742	Azaserine	50	97	Neg	Neg	---
746	Urethane	Neg	41	Neg	---	Neg
749	8-Azaguanine	58	62	Neg	---	---
750	Myleran	Neg	Neg	Neg	---	---
755	6-MP	50	Neg	Neg	Neg	Neg
762	HN2	55	>134	98	Neg	48
3053	Act. D	45	>175	103	64	---
3088	Chlorambucil	31	98	40	---	Neg
4728	2-Amino-thiadiazole	61	54	Neg	---	53
8806	Melphalan	75	200	162	27	38
10023E	Prednisone	Neg	Neg	Neg	---	Neg
13875	Hexamethylmelamine	Neg	Neg	Neg	---	Neg
14210	Merphalan	60	>200	278	50	---
17663	Alanine mustard	45	142	113	Neg	32
19893	5-FU	60	122	35	Neg	Neg
24559	Mithramycin	Neg	122	Neg	---	---
26271	Cyclophosphamide	80	>137	62	79	98
27640	5-FUDR	52	155	53	Neg	42
32065	Hydroxyurea	45	50	Neg	---	Neg
32946	Me-GAG	60	56	Neg	---	---
33669	Emetine	40	101	40	---	---
45388	DIC	67	54	50	34	Neg
49842	VLB	40	112	123	---	---
56054	Pseudourea derivative	61	108	>100	---	Neg
63878	Ara-C	94	121	33	---	Neg
67574	VCR	39	>135	89	---	---
77213	Procarbazine	45	Neg	25	---	Neg
82151	Daunomycin	58	127	>200	34	---
82196	BIC	200	195	89	34	---
95441	Me-CCNU	>200	---	47	50	>100
109724	5-HP	50	200	48	---	100
125066	Bleomycin	Neg	25	Neg	32	59
129943	ICRF-159	67	62	Neg	---	62
409962	BCNU	150	>200	113	47	30

Neg = ILS<25%

Table 5

Kinetic Parameters for Experimental Tumors

Tumor	Tissue Implanted	Site of Implant	Recipient Tumor Size	Td (Hrs)	Ts* (Hrs)	Tc* (Hrs)
AKR	Spontaneous	--	10^9-10^{10} cells	120	6	20
	1st passaged (thymus SC)		(early)	32	--	--
	1st passaged (thymus SC)		(Advanced)	72	--	--
	4th passaged		330 mg	33	8	27
B16	IP tumor	IP	11 days	40-60	8	16
	IP tumor	IP	14 days	40-60	11	18
B16	SC tumor	SC	600 mg	41	7	20
LL	SC tumor	SC	575 mg	70	8.5	19
L1210	ascites	IP	1 day	--	9	13
			5 days	13	12	16

* From labeled thymidine uptake studies
Td - doubling time, Ts - S-phase, Tc - Cell cycle time

Serial No. NCI-3578
1. Chemotherapy, CCNSC
2. Drug Evaluation Branch
3. Screening Section
4. Building 37

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Basic Studies for Drug Evaluation Utilizing Antitumor Screening

Previous Serial Number: Same

Principal Investigator: B. J. Abbott

Co-Principal Investigators: M. Baratta, R. Geran, N. Greenberg, D. Abraham

Cooperating Units: Immunotherapy and Immune Response Modifiers Section (J. Glynn); Drug Development Branch (J. Driscoll); Experimental Therapeutics (D. Cooney, J. A. R. Mead); The George Washington University, School of Medicine, Department of Pharmacy (W. R. Jondorf).

Man Years: Total : 5 1/2

Professional: 2

Other : 3 1/2 (includes only 1 full-time person, other staffing with part-time employees)

Project Description and Objectives

The Screening Section intramural laboratory conducts basic studies related to antitumor screening emphasizing the development of new and improvement of existing methods, and the detailed evaluation of drugs of interest to the Chemotherapy Program.

Methods Employed

Studies were conducted mainly in mice with transplantable leukemias L1210, P388, 5178YF, and first generation transplants of spontaneous AKR leukemia using established procedures and new procedures developed within this project.

Major Findings

1. L1210 Leukemia. Studies of structure-activity relationships among Emetine (NSC-33669) and a number of its structural analogs was completed (with Mead and Jondorf). Emetine was superior to the analogs included. The results of this study were recently published.

Of twenty drug combinations studied, the combination of Cytoxan (NSC-26271) plus Emetine (NSC-33669) is worthy of note. Marked additive toxicity and loss of therapeutic efficacy was observed. The basis for these results contraindicating the use of this combination will be explored.

The presumptive screen selected a number of analogs of cytosine arabinoside (NSC-63878, Ara-C) which were tested in detail. A study of the schedule dependency of these agents, limited because of the small amounts available for some, was initiated. Results to date indicate that none is superior to Ara-C using the divided dose-intermittent schedule (q. 4 hr., q. 4 days) that is optional for Ara-C. However, two agents, NSC-129220 (02, 2' - Cyclocytidine, monoacetate) and NSC-117614 (Cytosine, 1-B-D-arabinofuranosyl-,5'-(1-adamantane-carboxylate) were more than twice as effective as Ara-C when they were compared using daily (qd., Days 1-9) treatment. Both NSC-129220 and NSC-117614 produced substantial numbers of long-term survivors when given daily; a result which was not attainable with Ara-C. When given as a single treatment (Day 1), NSC-135962 (Palmo-ara-C), and NSC-135057, 1, 3-Bis (1-B-D-arabinofuranosyl-1, 2-dehydro-2-oxo-4-pynmidinyl)-2-thiourea, produced long-term survivors while Ara-C itself showed only borderline effectiveness and produced no survivors when given as a single treatment.

Comparison of NSC-82151 (Daunomycin) with its analogs was initiated. In preliminary testing to date, none has been superior to the parent compound.

NSC-141540, an Epipodophyllotixin derivative, demonstrated an ILS greater than 350% in preliminary testing. Confirmation testing has been scheduled to confirm this observation.

Although NSC-3053 (Actinomycin D) was reported to enhance L-Asparaginase activity (NSC-109229) in vitro, the combination was not more effective than NSC-3053 alone against L1210. However, this combination was more effective than NSC-109229 alone against L5178Y (previously reported).

Dr. John Driscoll, Drug Development Branch, synthesized the hydrochloride salt (NSC-133891) of Ellipticine (NSC-71795). Comparison of the efficacy of these materials disclosed the hydrochloride salt, which was soluble in water whereas bulk Ellipticine was insoluble in water, to have efficacy at fewer dose levels than NSC-71795 and to be toxic at lower doses than suspended bulk material.

Two experiments were scheduled to test for efficacy timed decomposition products of NSC-50741 B-D-Ribofuranoside, methyl 5-(2-chloroethylamino)-5-deoxy-2, 3-O-isopropylidene-, hydrochloride. Based on this experimental data, Dr. Driscoll is conducting a program to isolate the active "decomposition product".

2. P388 Leukemia. This tumor system was primarily utilized to confirm the activity of crystalline natural products.

3. 5178YF Leukemia. An L-Asparaginase resistant variant of 5178Y was developed for the study of potential inhibitors of asparagine synthetase. One such agent, NSC-13684 (Maleimide) was tested and found ineffective against both the sensitive and resistant lines. However, the compound did inhibit the normally elevated asparagine synthetase in the resistant tumor (with Cooney).

4. AKR Leukemia. Sixteen compounds which were previously found to be either active or inactive against the primary AKR tumor (with Glynn) were tested against the first generation transplants of the spontaneous tumor to establish the extent of correlation between these systems. The compounds were tested in either the optimal schedule and dose range previously determined for their testing in other tumor systems, or the schedule used in their prior testing against the spontaneous AKR tumor. Using an increase in lifespan of 50% as an endpoint, six agents were active in both systems, four were active against the first generation transplant and inactive against the primary disease, one was active against the primary disease and inactive against the first generation transplant, and five were inactive against both. This degree of correlation between the systems suggests that the first generation transplant could provide a useful prescreen to select candidates from among random new agents for evaluation against the spontaneous AKR lymphoma. However, the small number of agents in the current study were all known experimentally active antitumor drugs (mostly against L1210). A project for the screening of a reasonably large number of agents, including some without previously demonstrated antitumor effect, against early and advanced 1st generation transplants of AKR, and subsequent evaluation of "actives" against the sponteneous tumor is being initiated.

5. Other Studies. The effects of NSC-409962 (BCNU) on regenerating mouse liver was studied. Normally, regeneration occurred after surgical removal of up to 73% of the mouse's liver and is complete approximately eight days after surgery. The system represents a means of inducing rapid growth in vivo and for studying the effects of drugs on rapidly proliferating tissue. Tolerated doses of BCNU inhibited the normal growth of regenerating liver by approximately 30%. Light microscopy studies revealed that BCNU produced pyknosis of hepatocyte nuclei, margination of the hepatocyte nuclear chromatin, vacuolation of hepatocyte cytoplasm, dilation of blood vessels, reduced frequency of hepatocytes in mitosis, and increased proportion of hepatocytes with nuclei $\geq$ ten microns in diameter. Electron micrographs revealed that BCNU produced condensed mitochondria, endoplasmic reticulum with dilated cisternae, and enhanced electron-dense deposition along the inside of the nuclear membranes. The normal disc electrophoresis blood protein banding patterns were normal. The effects of BCNU were compared with parallel observations resulting from the administration of several drugs with previously reported modes of action. On the basis of this comparison it is suggested that two possible

modes of biological action of BCNU may be: interference with formimino transferase and interference with cytochrome oxidase.

In order to aid in the development of parenteral forms, the effects of anti-cancer agents which are markedly water insoluble but whose solubility in oil is suffuciently high to permit the administration of a therapeutic dose, an effort is being made to develop an oil in water emulsion solvent system which is physiologically acceptable to the mouse. Studies indicate that the oil in water emulsion (patterned after Upjohn Company's Lipomul formulation formerly available commercially) is well tolerated by the mouse when administered I.V. NSC-79037 (CCNU) when solubilized in the inner phase (oil) appeared to remain chemically stable and therapeutically effective when given I.P. or I.V. to mice with I.P. L1210.

The physiological disposition of NSC-114347 (Quinolinium, 6-amino-4-[3-amino-4-[[p-(4-pyridylamino)-phenyl]carbamoyl]anilino]-1-methyl-, p-toluenesulfonate, metho-p-toluenesulfonate) was studied using tritiated drug labeled by irradiation using the Wilzbach technique by Amersham-Searle. Radioactive material was purified by repeated chromatography in butanol: acetic acid: water, elution of the radioactive area corresponding to NSC-114347 with dimethyl sulfoxide, removal of the dimethyl sulfoxide by column chromatography on Cellex-P, elution, and evaporation. The purified material was judged to contain >95% drug corresponding to authentic NSC-114347. Following IP injection of $1x10^6$ cpm into mice, 65% was excreted unchanged within 24 hours. Over a 24 hour period, retention of radioactivity was greatest in liver, kidney, and lung in that order. Blood levels rose rapidly after injection and fell slowly suggesting the possibility of protein binding. In vitro dialysis studies confirmed this. Concomitantly, the A. D. Little group found that the drug formed a complex with lipid-like material. The latter observation was confirmed in this laboratory, and a complex with lipid extracted from monkey brain was easily demonstrated. Protein bound drug was readily converted to the lipid complex indicating that the affinity of the drug for lipid was greater than for protein. The nature of the lipid complex and its role in tissue-drug concentration is being studied.

Proposed Course

Continuation of studies related to the development of improved assays for drug evaluation, structure-activity studies, studies of combination chemotherapy, and studies devoted to the solution of special problems arising during formulation development, toxicological examination, and clinical trial of active antitumor drugs is anticipated.

Publications

See current report, Drug Evaluation Branch, Office of the Chief.

ANNUAL REPORT

PROGRAM ANALYSIS BRANCH

CANCER CHEMOTHERAPY NATIONAL SERVICE CENTER, CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

Chief, Program Analysis Branch	Barbara R. Murray
Assistant Chief	Rhoda Mancher
	Alan J. Greenfield
Head, Data Processing and Computer Systems Development Section	Sidney Richman
Act'g Head, Scientific Records Section	George F. Hazard
Act'g Head, Literature Research Section	Dorothy Algire
Head, Publications Section	William T. Soper

The Program Analysis Branch was transferred in July 1970 from the Office of the Scientific Director for Chemotherapy to the Cancer Chemotherapy National Service Center. The Laboratory and Clinical Trials Section was abolished when the staff dwindled to two, but some of its functions and responsibilities reverted to the Office of the Chief and remained within the Program Analysis Branch.

SUMMARY OF PROGRAM ANALYSIS BRANCH ACTIVITIES

Program Analysis Branch is working with Division of Computer Research and Technology (DCRT) in the design of an interactive chemical information system intended to assist laboratory chemists and toxicologists. The Branch is also investigating a number of commercial services with a view to augmenting staff resources. In FY 1971 the work of the Branch was supported by the following contracts: Chemical Abstracts Service (PH43-65-1021); Control Data Corporation (PH43-67-1356); Information and Communication Applications, Inc. (NIH-69-2236); NYU Medical Center (PH43-67-1336); John I. Thompson Company (NIH-69-2158); and, Vitro Laboratories (NIH-69-58).

Data Processing and Computer Systems Development Section made significant progress this year in handling Chemotherapy chemical and biological information. In the course of monitoring contractor operations to assure the regular processing and distribution of CCNSC information, the following major accomplishments took place:

- All CCNSC programs and data processing procedures were thoroughly documented.
- Screening data files were queried for a wide variety of scientific questions.
- A computerized plant information file was established and used for botanical queries.

An index to all CCNSC documentation is being prepared by machine to permit access to existing information in the CCNSC data processing system.

Chemical information handling was improved by speeding the registration of new compounds and improving the integrity of the file for non-structural information. CAS Registry Numbers are to be added to the file and thereby provide access to the literature. Polymers and coordination compounds are being machine-registered. While CAS continues to provide Substructure Search services, the feasibility of conducting searches in-house on the Selected Agents subfile is being investigated. Experimental, interactive chemical systems operating on the NIH reservation are being studied. The use of computerized techniques were continued to identify compounds in the Walter Reed antimalarial collection suitable for acquisition by the Chemotherapy Program. A procurement effort is in process to develop an extended chemical information system to mechanize the acquisition, shipping, and documentation of chemicals and to link the biological files with a state-of-the-art chemical substructure search capability.

Scientific Records Section significantly changed its file handling procedures, reflecting a trend toward methods and procedures best suited for input storage and retrieval of the large Chemotherapy chemical file. As a result of the Chemical Abstracts Service systems study in the Spring, 1970, a decision was made to use the CAS registry system for all duplicate checking during 1971. To do this, a new rough chemistry card was developed to facilitate large scale input and a large backlog was built up to allow smooth transition to the new procedure. Beginning in July 1970, this permitted checking for duplicates by structure only and systematic names were assigned only to non-duplicates rather than to all new input as in the old procedure. A semi-automated computer produced log was started as an aid to batch control of registry shipments and will be further developed by the DP&CSD Section.

As a further aid to file management, a new correction form was developed and used to synchronize corrections of all files. A major effort began to microfilm all card files in the Section for faster access and greater file security. The Section began active use of the Desk Top Analysis Tools (DAT) computer produced name and molecular formula indexes of the chemical file. These indexes were updated quarterly on a cumulative basis during the year.

Two major efforts were initiated in FY 1971 to double-check the CAS name files and allow more active future use. The first effort was a computer cross-check of supplier code, discreet code, and publication code against the CCNSC biological file. The second, begun late in the year, was to eliminate redundant and incorrect names. The computer structure file is being used to obtain CAS registry numbers, a valuable retrieval tool, as well as synonyms for all active compounds. Also, the machine registration of polymers and indeterminates was started to eliminate the former need to manually duplicate-check these classes.

Scientific Records Section cooperated with DCRT during the year on an experimental interactive substructure search system of future use to NIH scientists. The Section also continued its cooperation with Walter Reed Army Institute of Research (WRAIR) in exchanging information useful to the drug development

programs of each. A member of the WRAIR antimalarial program attended the CAS substructure search workshop for NCI and several searches were exchanged between the two organizations. The project to exchange compounds between the two files continued, using the machine-readable files of both groups.

The Literature Research Section filled approximately 80 requests for literature searches during the year. Information from the open literature, from Contract Progress Reports, and from specialized MEDLARS searches was obtained primarily for Decision Network review, for meeting FDA requirements for IND filing, and for the preparation of clinical brochures. The Section also assembled and distributed the quarterly Chemotherapy Program Statistics showing compounds entering the screen, the type and number of tests, the compounds passing each Decision Network step, and the compounds for which INDs are filed.

A recurring MEDLARS bibliography on cancer chemotherapy was initiated and a sample copy was evaluated. It is expected that this bibliography will be available when the MEDLARS II system becomes operational at the National Library of Medicine later this year. In addition, the Section has given special attention to perfecting the quality and frequency of the publication Cancer Chemotherapy Abstracts, and to improving the services of the Chemotherapy library.

Publications Section produced 6 issues of Cancer Chemotherapy Reports Part 1 in 1970, completing its 12th year of continuous publication. The Section also published two Chemotherapy Fact Sheets and cleared 160 contractor manuscripts for publication. Work is approaching completion on Volume 2 of CCR Part 2 and papers are in preparation for a second volume of CCR Part 3; neither Part 2 or Part 3 has been published since 1968. Increased attention is being focused on CCR as a series of publications, both to increase the frequency of Parts 2 and 3 and to clearly distinguish the editorial policies and types of information suitable for each CCR segment. This emphasis is one step that can be taken in anticipation of the future increase in publications that must result from an expanding cancer research program.

HONORS AND AWARDS

Barbara R. Murray was named Chairman of the COSATI Task Group on Chemical Information Systems.

The Scientific Records Section received a group Superior Work Performance Award citing its continued high quality of production despite a shortage of personnel.

PUBLICATIONS AND PRESENTATIONS

Murray, B. R. Data processing in chemotherapy program. Interface, April 14, 1970.

Murray, B. R. ADP support for the National Cancer Institute research program. Presented at the Seminar on ADP and Medicine, ADP Management Training Center, Civil Service Commission, Washington, D. C. on April 20, 1971.

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Satterlee, W., Thompson, W. L., Goldman, G., Gottlieb, M., Schwab, S., Block, J., and Murray, B. R. Automated monitoring of drug therapy. Presented at meeting of the American Association for Cancer Research in Philadelphia, Pennsylvania, April 1970.

Schein, P. S., Davis, R. D., Carter, S., Newman, J., Schein, D. R., and Rall, D. P. The evaluation of anticancer drugs in dogs and monkeys for the prediction of qualitative toxicities in man. Clin Pharmacol Ther 11:3-40, 1970.

Wright, S. E., Ting, R. C. Y., and Gallo, R. C. Transfer RNA methylase alterations in polyoma and spontaneous malignant transformed mouse cells in culture. Clin Res 27:615, 1969.

OFFICE OF THE CHIEF

Program Analysis Branch

I. Systems Management

The system for reporting costs of drugs shipped to cooperating investigators was completely rebuilt this year because of budgetary restrictions on these shipments and the consequent necessity for frequent close monitoring of these costs. Ceilings were established for the supported cooperative groups. To provide for daily transaction input, the Conversational Programming System (CPS) at DCRT was used to code and edit drug shipment requests. Monthly reports of shipments to cooperative groups by drug and by investigator were implemented, which showed ceiling and percent of ceiling used. A general package of routines was created for producing quarterly reports of interest, and IRS (Inquiry and Reporting System) was used for specialized reporting. Experience with the system was good and the rapidity of data acquisition allowed prompt responses to requests. The major problem in establishing the new system was to fix ground rules for assigning costs to given groups. An on-line investigator name file allowed tight control of this problem.

The active investigator file was transferred to CPS to allow the rapid interrogation and updating of the file needed to maintain adequate control over assignment of costs of drug shipments to cooperative groups. Plans are in process to convert the CDC 3600 INFOL name and address file of investigators to run at DCRT.

II. New Projects

The Assistant Chief is in charge of a small group working on new projects. During the past year, this group concentrated on the extension of the CCNSC data processing system, which processes mainly primary testing results, to encompass the secondary evaluation carried out on compounds of special interest to the program. The original CCNSC data processing system was conceived solely to handle results of primary screening of potential antitumor agents and its major file order is by single drug. At the time it originated, no thought was given to automating the more sophisticated and extensive secondary testing. In the intervening years the role of CCNSC changed but the function of the computer system did not. The file now contains primary screening information on 317,000 materials and it exists both as an historical repository and to report screening on a regular basis. Approximately 30,000 fermentation products were not added to the file because they were inactive. The system underwent major reorganization when it was moved from the IBM 1401/1410 to the IBM series 360.

A logical extension of the single drug regimen or schedule dependency testing is a carefully designed system of experiments to determine the

effects of active compounds in combination. These experiments approximate the clinical use of drugs and are an attempt to predict in the rodent the most effective nontoxic antitumor therapy before further tests in dogs and primates.

With increased emphasis on therapeutic trials in the rodent, introduction of surgical adjuvant therapy, and extension of the AKR system, it was apparent that a different ordering of the combination chemotherapy file would permit handling a broader range of secondary evaluation data. Separation of control information from the test data will permit rapid queries to determine in what combinations a compound has been tested. Retrospective information will be available as a management tool in planning further experiments. Experiments at NCI will be coordinated with those performed at other testing laboratories. This work is being carried out in conjunction with Southern Research Institute, which is cooperating in the analysis and statistical design.

III. Clinical Data Processing

Responsibility for clinical data processing was removed from PRA at the beginning of FY 1971. In the interim between this action and establishment of an operating center of responsibility, PRA, in the Office of the Chief, continued to provide service to program clinicians.

The maintenance of the Masterfile was continued. To avoid duplication between the on/off study and Master Files, the protocol and study drug fields were eliminated from the master file and data on complications substituted. Data exists for approximately 9,000 patients: 8,333 NCI-Bethesda patients, 381 Baltimore Cancer Research Center patients, and 14 Veterans Administration patients. This file continues to provide data by various levels of classification, principally by diagnoses.

The on-study system appeared useful to clinical investigators as evidenced by the addition of data on 650 patients. Because of the difficulties with mark-sensing equipment before DCRT acquired an Optical Character Reader (OCR), the NIH Conversational Programming System (CPS), a time-sharing system with fairly sophisticated I/O capabilities, was used for editing data from the mark-sense sheets as input to the on-study file. The increase in speed and decrease in error rate more than compensated for the increased operating cost.

Later in the year a prototype of a terminal-oriented on/off study system was developed on CPS, which entailed the creation of a method to maintain unique disk addresses for each patient number/protocol number/randomization number combination. Early experience indicates that this will be a valuable interim solution to moving the data closer toward the investigator. A really sophisticated system must await the appearance of true teleprocessing equipment able to take full advantage of the speed and record-handling capability of third-generation computers. The cost of the interim system, however, is several times that of the original system because of the need for on-line storage and the inefficient nature of

time-sharing. On the other hand, as in the mark-sense coding program, error rate is markedly decreased and speed greatly increased.

The Interval Summary Sheet was discontinued at the request of the Chief of the Medicine Branch. The laboratory data weekly summary sheet continued at staff request and was successfully interfaced with the Beta system in the Clinical Center Clinical Pathology Laboratory.

The off-study section of the on/off study file was implemented during the latter half of the year. Use increased slowly since the sheet is a compromise between the needs of a number of investigators. At present the on-study file contains on-study data on 66 Veterans Administration, 425 Leukemia Service, 524 Solid Tumor, and 160 Baltimore Cancer Research Center patients, and off-study data on 21 Veterans Administration, 89 Leukemia Service, 19 Solid Tumor, and 9 Baltimore Cancer Research Center patients.

Protocol accrual reports were prepared and distributed weekly. A number of improvements have been suggested by the Solid Tumor and Leukemia Services to reflect the dichotomy in their criteria for positive response. At this writing, several off-study and protocol summarization reports are in process of analysis and programming.

A life table program that prints variable format tables, and median survival times, and graphs single or multiple cohorts, was produced this year in a format allowing interface with the on/off study system to produce survival analyses of the data.

The Drug Fact Sheet was converted to a booklet of pertinent abstracts distributed to all nursing units and Clinical Associates. The books were very popular, 50 copies being distributed within two months of their availability. It is planned to update the booklet and add new drugs as information becomes available, and to review and republish the booklet annually. This work is done in conjunction with the Medicine Branch and the Clinical Center Pharmacy.

The Cell Separator project was completed this year with the addition of HLA typing to the Donor/Recipient file, and a report of Net Histocompatability Ratio expressing mathematically the degree of match between a prospective leukocyte donor and recipient. The system was produced by Information and Communication Applications, Inc. (ICA) and accepted with satisfaction by the investigator requesting it, and has been used to formulate approximately 25 queries of the Cell Separator data base. In addition, the basic retrieval program (Generalized Select) was successfully adapted to interrogate other data bases.

The L-asparaginase report was discontinued after new input became minimal but the file is still available for retrieval.

PRA will continue to provide support in clinical data processing until other mechanisms are effected for these functions. Our experience in

this field and the knowledge that the Clinical Center is now in the process of automating much of their medical records and data handling, leads us to believe that the program can best be served at a central, supra-institute level. With this approach, the equipment, personnel, and know-how required would be available and the tremendous quantity of duplicated effort in each investigator's office would be eliminated. NIH could then begin to approach the state-of-the-art in teleprocessing and the research effort will be greatly augmented and strengthened. However, careful and exhaustive analysis and planning will be required to produce the level of flexibility necessary for successful implementation of such a system, while maintaining the data bases in a format which will be interchangeable and amenable to analysis by common procedures.

DATA PROCESSING AND COMPUTER SYSTEMS DEVELOPMENT SECTION

Program Analysis Branch

This Section is responsible for the management and improvement of the operating data processing system for the Cancer Chemotherapy National Service Center (CCNSC), contributes systems analysis support in monitoring the Chemical Registry System operated by the Chemical Abstracts Service (CAS), and participates in the effort to develop an extended Chemotherapy chemical information system.

I. Contract Operation

A. Under contract NIH-69-58, Vitro Laboratories furnishes data processing support for the Chemotherapy Program. This contractor's primary task is to receive antitumor screening data from laboratories worldwide, edit and keypunch input (as required), initiate and monitor the computer processing of these data, and ensure the timely dissemination of the results to qualified investigators. Over 132,000 anticancer experiments were reported during 1970. Other contract activities include (1) disseminating regular statistical reports on CCNSC data; (2) preparing input for drug schedule dependency plots; and (3) programming to maintain the CCNSC data processing system, query the various CCNSC files, and support the Clinical Trials area and the Endocrine Evaluation Branch. A new EEB data processing system was developed during the year. Emphasis was placed primarily on: (1) attaining regular production status and smooth operation of the CCNSC data processing system; (2) documenting thoroughly the programs and data processing procedures so that the system would be contractor-independent; (3) improving the efficiency and accuracy of the system; and (4) developing more sophisticated data handling and analysis techniques.

B. Under contract PH43-65-1021, the Chemical Abstracts Service (CAS) provides computer programs, hardware, and staff support (at its Columbus, Ohio facility) for the registration of chemical compounds new to the NCI screen.

II. Highlights of the CCNSC Data Processing System

A. Regular production status at NIH's computer facility has been continued. Input of data, correction of errors, and mailing of a variety of recurring reports now proceeds smoothly. File updates are currently run biweekly rather than weekly with a saving of $30,000 per year.

B. Eighty-one operating procedures (1001 pages of text) were completed along with complete documentation for 25 recurring programs totaling some 3,000 pages of text, flow charts, and program compilations. Supplementary documentation, not counted in these totals, included

a CCNSC Run Book and a Handbook of I/O formats. In short, the CCNSC data processing system is thoroughly documented, permitting the transfer of the data processing system to another contractor, if desirable.

An index to the CCNSC data processing system is being developed by machine to serve as a reference for CCNSC and contractor staff. It contains citations covering all CCNSC documentation, permits ready access to existing information, and insures that any change in the processing of data is reflected in all parts of the system.

C. Some 44 programming changes were made to the CCNSC programs to improve communication between the system and the users. The Materials Classification code now indicates the status of a compound in the linear array. Test status codes were machine-assigned to 200,000 test records. Codes were assigned by machine to 22,000 compounds to identify those without a sufficient quantity available to complete testing. Statistical reports were computer-generated, providing information not previously available, such as the number of materials tested by type during a given period.

D. File queries were an invaluable aid in accessing information, providing a spectrum of data (in 13 queries) that would be impossible to obtain by manual scanning of Screening Data Summaries. A query was made to determine the relation between confirmed activity in cell culture and activity in the leukemia L1210 system. Another query identified quarterly production of plant fractions by fractionator and the test system in which they were tested. Programs were written to query the Plant Header File to produce listings of plants (ordered by family and botanical name) collected in specific foreign countries.

III. Highlights of Chemical Information Activity

A. Through March 15, 1971 the Chemical Abstracts Service has registered 156,861 compounds, built a nomenclature file containing over 200,000 chemical names, and established a Fragment Bit File used as a preliminary step in substructure searching.

Registration is now conducted every two weeks rather than every three weeks to speed the processing of compounds through the Branch. An interactive computer system is being developed to track the flow of chemical information, by compound, during the registration process. Cumulative updates of our name and molecular formula indexes are produced quarterly. The CAS Registry Number, widely recognized as the key to chemical information interchange, is being added by machine methods to the NCI Bibliography File. Some 5,000 polymers and coordination compounds, previously registered manually, are now being registered by computer.

B. A week-long workshop was conducted at CAS to familiarize NCI chemists and systems analysts with the CAS Substructure Search system and to gain practical experience in query coding. Over 180 substructure searches have been performed on the Chemotherapy structure file. A file of nondiscreet Selected Agent structures was delivered to NCI for a full scale operational test of DCRT's interactive search system. A complete subfile of SAC structures with a corresponding subfile of fragment codes is being prepared to permit PRA to operate the CAS Substructure System at DCRT and abandon the remnants of the optical coincidence (peek-a-boo) system used for fragment searching.

C. CAS continued to provide essential nomenclature consultation and began a thorough review of all NCI bibliographic information, including the addition of synonyms for Selected Agents and the elimination of redundancy in systematic nomenclature.

IV. Extended Chemotherapy Chemical Information System

The Chemical Abstracts Service successfully completed a study of a functional design for an extended Chemotherapy chemical information system which laid the groundwork for a procurement effort to develop a detailed system design to: (1) mechanize and improve the present chemical workflow; (2) link the chemical and biological files to facilitate structure-activity studies; (3) unify the chemical registration and nomenclature effort into a single, modern computer-based system; (4) provide state-of-the-art substructure search facilities; and (5) machine-produce many forms and documents now completed manually, such as Materials Shipping Lists and chemistry cards.

V. Other Significant Highlights

A. The PRA remote job entry computer was used to enter over 10,000 jobs in DCRT's facilities this year. A console was added to the model 20 to provide complete control over the submission and reception of jobs.

B. The Section constantly investigates new methods of data entry and handling. Devices given particular attention this year were remote time-sharing terminals, graphics display devices, and computer output microfilm hardware. Other offline equipment such as microfilm retrieval systems were also studied. Every effort is made to incorporate state-of-the-art developments into the NCI operation.

LITERATURE RESEARCH SECTION

Program Analysis Branch

The Literature Research Section serves as an information center for the Cancer Chemotherapy Program and the NCI staff. Information obtained by the Section is used in Decision Network review, in writing clinical brochures, and in meeting FDA requirements for IND filing. High priority continues to be given to literature searching for the Linear Array.

I. Literature Services

Information from the world's literature on cancer chemotherapy and related areas, and from the unpublished data of the Cancer Chemotherapy Program, is provided in the form of abstracts, bibliographies, data compilations, and copies of articles. Every effort is made to fill requests by using existing services such as MEDLARS and AIM-TWX to avoid duplication of effort.

Eighty-one requests for literature searches were filled during the year ending April 1, 1971. Comprehensive bibliographies were prepared for such agents as streptozotocin, adriamycin, 5-azacytidine, concanavalin A, and BCNU. Extensive clinical searches included: the chemotherapy of bronchogenic carcinoma; the chemotherapy of colon carcinoma; clinical studies with L-asparaginase; busulfan in clinical cancer; and therapy of meningeal leukemia. The Section also filled requests for over 100 previously prepared bibliographies and distributed copies of over 4000 articles.

An extensive file of the world's published literature on chemotherapy and a collection of Contract Progress Reports is kept by the Section to aid in its work. The Section is also responsible for maintaining the Chemotherapy Library, a collection of books, journals, films and current Progress Reports for the use of the Chemotherapy Staff. Additional shelving was acquired this year and $8,000 was spent on books and journal subscriptions. Over 1900 journals were circulated.

The preparation and distribution of agent folders for the Decision Network Meetings was continued during the year. Forty folders for each of 47 drugs were prepared and distributed to members of the committee in advance of the meetings. Each folder contained the chemical structure and systematic name of the compound, an analog search, a literature search, a summary of clinical data if in clinical trial, and Decision Network questions and answers.

The Section was also responsible for assembling and distributing the Chemotherapy Program Statistics Reports. These quarterly reports include statistics on the number of compounds received, the number of tests performed, the number of confirmed active compounds in the primary screen, and lists of compounds passing each Decision Network step. Drugs for which INDs are filed are also listed.

In response to the needs of the Chemotherapy Program for information on new drugs, the Section initiated and procured this year a recurring MEDLARS bibliography on new and potential antineoplastic agents. Copies of the bibliography are distributed each month to members of the Drug Development Branch. Efforts towards procuring a comprehensive recurring cancer chemotherapy bibliography from the National Library of Medicine will continue when the MEDLARS II system becomes operational at the Library.

To further serve the Chemotherapy Staff, the Section will be testing the value of a selective dissemination service using the Automatic Subject Citation Alert (ASCA) tapes of the Institute for Scientific Information. The weekly bibliographies will keep the staff abreast of the latest developments in cancer chemotherapy and permit the Section to keep its files up-to-date.

II. Contract Operations

The Literature Research Section monitors two service type contracts, one for the preparation of Cancer Chemotherapy Abstracts and the other for microfilming and indexing the chemotherapy literature.

Cancer Chemotherapy Abstracts, a comprehensive bimonthly bulletin, provides abstracts of the world's literature in the field of cancer and supplies the Literature Research Section with hard copy of all the articles abstracted and cited. Delays in production have occurred in recent months but every effort is now being made to bring the publication into phase. Monitoring continues to be directed toward improving the agent indexes as well as the overall quality of the service.

The George Washington University project was designed to provide for rapid scanning and easy retrieval of the literature of Cancer Chemotherapy Abstracts. Filmed copies of the 1960-1964 literature, a reader-printer, and a cumulative author index have been provided. Work on the cumulative agent index is nearing completion.

PUBLICATIONS SECTION

Program Analysis Branch

The Publications Section is responsible for all publications of the Chemotherapy Program, clearance of contract manuscripts, and editorial assistance to the Chemotherapy staff on a time-available basis.

Publications Section directs the preparation of Cancer Chemotherapy Reports (CCR), the principal scientific journal developed under the Chemotherapy Program from the original recommendations of the Chemotherapy Committee of the National Advisory Cancer Council. Until 1968, CCR was a single journal but in that year it was expanded to 3 parts. From its inception, CCR (and CCR Part 1) has served the prompt reporting of original contributions in clinical and experimental studies of cancer chemotherapy, including complete works, preliminary investigations, commentaries and reviews, symposia, and letters pertinent to published articles. All candidate manuscripts are reviewed by 2 or 3 investigators familiar with the subject matter and the final consideration is made by the Scientific Editors. CCR is now in its 13th year of continuous publication with a total to date of 85 issues, including 2 issues of Part 2 and 1 issue of Part 3. In 1970, there were 6 issues of CCR Part 1 (Volume 54).

In 1969, it was decided that CCR Part 1 would publish the joint clinical discussions of the NCI Baltimore Cancer Research Center and The Johns Hopkins University Hospital on the management of patients receiving cancer chemotherapy. One of these, concerning a patient with Hodgkin's disease, was published in the June 1970 issue of CCR Part 1. Another, on the management of hypercalcemia complicating malignant disease, is in the editorial process for publication in the near future.

Beginning in January 1968, Cancer Research ceased publication of its Part 2 supplement, which contained chemotherapy screening data from the NCI and elsewhere, and related material prepared under the auspices of the Cancer Chemotherapy National Service Center. Arrangements were made to continue publication of this information in CCR Part 2. To date, only one volume (CCR Part 2 Supplement, Volume 1, Numbers 1 and 2, 1968) has appeared but a second volume containing 9 papers is scheduled for publication later in 1971. It will include a new statement of editorial policy for CCR Part 2 and guidelines for preparing manuscripts, as well as introductory remarks to clarify any misunderstanding of the relation of Part 2 to the other members of the CCR series. It is planned to continue and increase the frequency of CCR Part 2 publication subject to the availability of time and personnel.

CCR Part 3 (Program Information) also was established in 1968 to publish protocols and clinical brochures which are inherently not reviewable but are of interest to chemotherapists. A single issue (Volume 1, Number 1, 1968) has been published. Another issue, containing papers accumulated since 1968 and the presentations made at the First Joint Working Conference NCI Chemotherapy Program, December 16-18, 1970, in Annapolis, Maryland, is planned for FY 1972.

Table 1.--Summary data on the CCR Publications Series

	CY 1967	CY 1968	CY 1969	CY 1970	CY 1971 (Jan 1-Mar 31)
Manuscripts Received	125	107 (Part 1)	78 (Part 1) 4 (Part 2) 6 (Part 3)	122 (Part 1) 5 (Part 2) 1 (Part 3)	31 (Part 1) 3 (Part 2) 5 (Part 3)
Manuscripts Accepted	87	53 (Part 1)	63 (Part 1) 5 (Part 2) 6 (Part 3)	68 (Part 1) 4 (Part 2)	21 (Part 1)
Issues Published	7	7 (Part 1) 2 (Part 2) 1 (Part 3)	6 (Part 1)	6 (Part 1)	1 (Part 1, in press)
Pages Published	561	775 (Part 1) 539 (Part 2) 217 (Part 3)	408 (Part 1)	492 (Part 1)	97 (in press)
Reviewers	143	132	132	140	86
Printing Costs[a]	$13,462 ($24/pg)	$22,302 (Part 1) ($28.77/pg) $ 7,877 (Parts 2 & 3) ($10.41/pg)	$11,813.82 (28.95/pg)	$14,445.17[b] ($29.36/pg)	Not available

[a] Costs of GPO contract with Publications Press, Inc., Baltimore, Maryland.

[b] Actual cost of 4 issues + estimates of 2 issues (final costs presently unavailable).

CCR is available without cost to qualified medical groups, physicians, and libraries; it is for sale to others by the Superintendent of Documents, Government Printing Office. The approximate distribution, which varies with each issue, is as follows:

3600	Foreign and Domestic Free Mailing (includes ca. 200 in NIH plus storage copies for Section reserves)
87	GPO files
500	Superintendent of Documents (for paid subscriptions)
367	Superintendent of Documents (for Depository Libraries)
Total 4554	

Comparative data on the CCR series of publications are shown in Table 1. The manuscripts submitted and those that are accepted are reported monthly to the Editorial Office of the Journal of the National Cancer Institute, which in turn distributes this information in their monthly reports.

In addition to the regular journal series, a variety of other publications are edited and prepared in camera-ready copy by Publications Section. The Chemotherapy Fact Sheet was established in 1968 to disseminate information on new antitumor drugs to members of cooperative groups and other chemotherapists who were formerly provided such data in frequent meetings. Recent ones include "1-Aminocyclopentanecarboxylic Acid (NSC-1026)," published July 1970, and "Hexamethylmelamine (NSC-13875)" (February 1971). Five Fact Sheets have been published and they are continuing to receive favorable response with many requests for copies.

Another type of special publication is illustrated by the "Proceedings of the Chemotherapy Conference on Procarbazine (Matulane: NSC-77213) Development and Application" held at NIH on March 13, 1970, which is currently in preparation for publication later this year.

Publications Section is also responsible for maintaining the free mailing list for Cancer Chemotherapy Abstracts (CCA). This list is now being screened to determine the eligibility of recipients under the latest policies governing free distribution of Government publications. Another function of the Section is the coordination of clearance of contract manuscripts for publication. In 1970, 160 of these manuscripts were cleared compared to 197 in 1969. During the first quarter of CY 1971, 36 manuscripts were cleared.

In the coming year, Publications Section will continue its present program and expand it to meet the increase in technical publications which will undoubtedly occur with a greater national emphasis on cancer research. CCR Part 1 will continue, but in a newly established format and cover design. Hopefully, we will work toward improving the journal contents and the frequency and speed of publication. The Section plans, if time and personnel are available, to

produce more issues of Parts 2 and 3 and to clearly establish them as important segments of CCR. Of course, the expected upsurge in the literature of cancer chemotherapy will require similar attention to our special publications (Fact Sheets and Proceedings) and probably to new categories of informational material.

SCIENTIFIC RECORDS SECTION

Program Analysis Branch

The Scientific Records Section (SRS) provides supporting services and information to members of the Chemotherapy staff in nearly every phase of the development of chemotherapeutic drugs.

The Section prepares and maintains the master structure file and several cross-reference files on each drug. It indexes and organizes new compounds into files suitable for retrieval by name, NSC number, molecular formula, and chemical substructure. Section personnel also prepare shipping lists for the transmittal of compounds to screeners and coordinate the various special shipping instructions from CCNSC for certain categories of agents.

As compounds progress in the screen, the Section checks and corrects inputs and outputs for various printed information and control tools used by Chemotherapy scientists to follow the progress of drugs of interest. This information is provided as lists of chemical analogs, lists of active and synthetic natural products, and priority lists of compounds to be synthesized. Other information services, such as reproduction of chemistry cards and screening data summaries, and the retrieval of individual compounds, are offered for use in routine drug processing and evaluation.

The Section is responsible for performing substructure searches and developing new techniques and formats to make this information more useful to Chemotherapy scientists. During the past year, the computer search system developed at Chemical Abstracts Service (CAS) was used exclusively for all large substructure searches. In addition, the Section has investigated methods to make the system more economical and responsive to the needs of the Chemotherapy Program. The Section analyzed certain portions of the CAS system in terms of our needs and cooperated in the development of an on-line search system at the Division of Computer Research and Technology (DCRT).

In the clinical area, SRS provides services by punching and proofing patient admission and discharge data for input into a clinical master file. It also is responsible for sorting and mailing agendas, minutes, and other documents of interest to chemotherapy investigators. The Section has discontinued its support of input used in clinical drug cost reports and redirected the effort to improving chemical information capabilities.

I. Accession and Drug Processing (Stage I)

The Section processed 8521 compounds this past year, which required: (1) hand drawing of each structure; (2) punching of a flexowriter tape of card header information for each compound; (3) production of suppliers lists for acknowledgment of compounds; (4) production of shipping lists to serve as drug transmittal documents and computer inputs; and (5) input of name and structure to the CAS-NCI computer file.

To take advantage of the CAS computer registry system for duplicate checking, a new 10 X 8 rough chemistry card was developed by Drug Development Branch (DDB) and PRA to give a more efficient format for keyboarding structures and nomenclature at CAS. The lag between CCNSC accession and input to the CAS structure file was then eliminated and a large backlog of new compounds built up in June 1970 to ensure the continuity of workflow to CAS, suppliers, and screeners. This allowed use of the CAS computer registry system in FY 1971 to duplicate-check by structure only, eliminating the intermediate naming required in the previous system. During the year, names were given only to nonduplicates as shown by the registry system. About 20% of the new accessions were duplicates and an equivalent amount of nomenclature chemist time was saved by DDB. Since the flow of information to CAS was divided into 2 parts; i.e., structures for all accessions, and names, structures and ancillary information for nonduplicates, the Section developed a batchlog for each shipment using the on-line NIH computer text editor (WYLBUR). This log employs concepts developed in the CAS systems study plus working experience and is being further developed by the Data Processing and Computer Systems Development (DP&CSD) Section, PRA, using more advanced techniques. The working knowledge gained by SRS in computer chemical handling techniques will be a valuable asset when the proposed systems design and implementation of a new Chemotherapy Chemical Information System take place.

A new correction form was developed during the year by DDB with SRS consultation to centralize the correction of files and to give more accurate counts of such transactions. The form has been very helpful in keeping the various computer and card master files and subfiles synchronized. It has also systematized error correction of the CAS files, a matter of increasing importance because of the change to active rather than archival use.

II. File Maintenance

In addition to the new standard correction form, two major improvements in file maintenance were made. The first was the use of a computer program developed in cooperation with the DP&CSD Section to compare certain data elements in the CCNSC biological file and the CAS-NCI nomenclature files. For a given NSC number, the supplier code, publication code, and discreet code were checked against one another and appropriate error messages printed for all discrepancies. This allowed automatic correction of these important data elements in both files, and will ultimately permit more active use of the CAS bibliography file of names and molecular formulas as an on-demand print file, with the discreet and publication codes being used as security indicators as they are now used on the flexowriter. This cross-checking process will be used in the future as one of many processes for maintaining the high standards of file integrity needed by CCNSC.

The second major file maintenance improvement was the study and subsequent agreement with GSA to microfilm the various ancillary card files

maintained by the Section. The master card file, rough chemistry card file, and CAS edited file, and the old fragment code breakdowns will ultimately be filmed under this plan. Only the master card file will be retained as paper, and the other card files will be destroyed after a study of the quality of microfilm. The space now occupied by these files will house microfilm equipment, computer printout binder cabinets, and possibly an experimental terminal for the DCRT substructure search system. Hopefully, these steps will facilitate access to our files by qualified users and, at the same time, provide archival protection for the microfilmed copies. It is conceivable that microfilming could be an intermediate step toward a large scale chemotherapy microfilm system, if use warrants it.

III. Substructure Search

Substructure search is used in structure-function studies by the Drug Evaluation Committee (DEC) and at Decision Network 2 of the linear array. In addition, it is available as a retrieval tool for interested Chemotherapy scientists and, on a more limited basis, to qualified outside requestors. The Section converted entirely to the CAS substructure search system for master file searches and used it for 83 searches plus 60 updates of previous searches of recurring interest. In addition, 35 searches of the SAC or Selected Agents file were made for the DEC and other requestors using an optical coincidence system. The staff of SRS concerned with substructure search, plus others in CCNSC, attended a 1-week instruction session at CAS on the use of the system. During the year the SRS staff assumed an ever-increasing volume of search coding and used the remote job entry facilities of DCRT to edit their questions prior to sending them to CAS.

As part of a PRA project, the Section actively cooperated with the Heuristics and Data Management Branches of DCRT in development and implementation of various chemical search systems. Representatives of Data Management Branch attended the CAS course on substructure search and cataloged all the CAS search programs at DCRT. Two members of the Heuristics Branch, who are developing programs to perform structure manipulation, received PRA assistance and were provided various CAS utility programs, subfiles of published chemicals, and user feedback of use in their programs. Of particular interest to SRS was an on-line interactive search program based on the CAS connection table and using the graphics capabilities of the NIH PDP-10 computer. Using a file of 2500 nondiscreet selected agents, SRS aided DCRT in improving this program from the viewpoint of user needs and convenience. A suitable PDP graphics terminal will be made available to PRA during the coming year to continue use of this system and its development.

In addition to the above work, SRS has initiated research on possible improvements in the CAS search system, primarily an analysis of the efficiency of the prescreening step of the CAS search in terms of NCI needs. It is hoped that this analysis will reduce the future costs of searches.

The quality of search available and the ease of input has risen considerably with the change to computer methods. The rapidly growing literature in the field of computer handling of chemical structures leaves no doubt that sophisticated techniques for synthetic pathway analysis, toxicological evaluation, and complex structure-activity analysis will become available to investigators in chemotherapy. The experience now being gained by SRS in one of the more sophisticated structure retrieval tools is an excellent beginning in the use of these methods.

IV. Information Services

In addition to file building and on-demand searching, the Section is responsible for preparing input and checking output for several mechanized information systems.

Input is prepared to produce lists such as the Materials of Interest (MOI), Confirmed Active Plants (CPAM), the Selected Agents List (SAL), and the Quantity Not Sufficient (QNS) listing. These documents serve as reference tools for Drug Evaluation Branch (DEB) evaluation of natural products, active synthetics, and agents of requiring an additional supply. They are also used as reference documents by screeners and other chemotherapy investigators.

Biological testing output for natural products is checked and separated into groups for transmittal to the proper DEB staff members. Error outputs for shipping lists and control packs are also researched and sent back to the data processing contractor for correction. Printed output of the QNS was discontinued in favor of listings from the CCNSC tape file by request. This step was taken after a study of the QNS workflow was made by the data processing contractor at the request of PRA. The input procedures were streamlined, with SRS continuing to serve as the focal point.

The scheduling of the Materials of Interest list (MOI) for confirmed active natural products was changed to synchronize with its larger companion document, the Confirmed Active Plant Listing (CPAM). The full body of the report is now published quarterly instead of monthly, with new additions and modifications printed in the intervening two months. A large backlog of Screening Experiment Analysis (SEA) listings had accumulated with the conversion to the new CCNSC biological data processing system. These were all researched and resolved during the last part of 1970 and are now current. All SEA listings from July 1968 to June 1970 will be microfilmed according to specifications worked out with DEB.

In addition to cooperating in the production of the routine quality control documents, the Section developed several other types of information aids during the year as part of our effort to make file information more readily available to the individual scientist. The first was an analysis of ring systems contained in active compounds, showing

the ring system, name, and breakdown of totals by degree of activity. During the year KWIC computer indexes were generated for all large substructure searches run using the old manual and new computer systems. These will be expanded to indexes of the distribution of various classes of compounds in the file, with a separate breakdown for active and nonactive compounds. Further computer analysis of the chemical file, as compared to other files, will be done in the coming year in the hope of detecting leads to the nature of chemicals having antitumor activity.

The Section also continued to process its normal load of routine requests for xeroxing of chemistry cards and screening data, production of flexowriter listings, retrieval of synonyms and all the other daily requests that are expected of a records section. The use of computer produced molecular formula and name indices of our file that were generated under contract by CAS have taken an increasingly important role in this type of service. Computer output of name or structure on-demand will greatly facilitate this process when it is available in the future. To improve record keeping and provide centralized control of requests for SRS services, the Section has adopted a joint request form with DP&CSD Section. Hopefully, the statistics derived from the use of this form will allow better allocation of PRA resources.

In anticipation of possibly increased demands for literature retrieval by Chemotherapy investigators, SRS participated in two new ventures. The CAS registry number, which is a unique identifying number for all compounds abstracted by CAS since 1965, will be published in the literature beginning in 1971 and promises to be a valuable retrieval tool for individual chemicals. Using machine methods developed in cooperation with CAS, the registry numbers are being obtained for all NCI compounds which are on the CAS master file. The CAS registry numbers for SAC compounds will ultimately be published with pertinent synonyms as a literature retrieval aid to the Chemotherapy community.

The second project is an attempt to provide faster, more comprehensive access to the literature through a pilot project initiated to test the various computer Selective Dissemination of Information (SDI) systems. In this type of service, an individual's interests are identified by key words and chemical structures which are used to search machine-readable files derived from the primary literature. This project is a cooperative one between DDB and PRA to investigate costs, develop an optimal set of terms for managerial, chemical, and biological areas of interest, and study output for usefulness. We hope that after initial trials are completed, this service can be more widely available as an aid to acquiring new compounds, conducting searches of past and present technical literature, and promoting better awareness of developments in the field of Chemotherapy.

V. Future Plans

The Scientific Records Section will continue its efforts to improve operating procedures and access to the files. The use of microfilm

computer techniques and the production of specialized indices will play a larger role in providing this improvement.

The most significant factor during the next 2-3 years, in terms of upgrading and streamlining services, will be the gradual conversion of the chemical information system to computer methods to allow expedient handling of such a large file. As one of the main links in the CCNSC chemical information system, the Scientific Records Section will be heavily involved in this effort, both in developing new procedures and new methods and in monitoring any system implementation.

Efforts will continue during the coming year to effectively use the present CAS contract to perform substructure searches, and to continue building our computer structure and nomenclature files, maintaining a file quality and integrity that will allow easy conversion to future machine processable formats.

SUMMARY REPORT

ASSOCIATE SCIENTIFIC DIRECTOR FOR CLINICAL TRIALS

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

The past year has seen the continuation of a shift with major emphasis and effort being directed toward the common solid tumors, carcinoma of the breast, lung, and colon. It is of interest to review the Annual Reports of the last few years in order to appreciate fully the changes which have come about. Acute and chronic leukemia, lymphomas, particularly Hodgkin's Disease and Burkitt's Lymphoma, and a miscellaneous group of solid tumors have received a great deal of attention both in attempts to improve antitumor therapy and in studies concerned with the pathophysiology of these diseases. Relatively little work was devoted to the major tumors until last year when the NCI-VA Medical Oncology Unit emphasizing clinical trials in bronchogenic carcinoma was established.

Programs were initiated to improve supportive care of patients with special emphasis on blood component therapy. The development of the NCI-IBM Cell Separator was a critical step in this area particularly in granulocyte procurement and the use of HL-A matched platelets and granulocytes is becoming an integral part of the management of patients with cancer. The protected environment program began with a small unit which however led to the development of a highly satisfactory portable germ-free room employing the laminar air flow principle. After several years of discussion a protocol is now in effect designed to define the role of this environment in the treatment of cancer. The effect of reducing the endogenous microbial burden on the incidence of infection in leukopenic patients is also being evaluated. Finally, a variety of combination drug regimens have been evaluated notably in acute leukemia and Hodgkin's Disease and have shown remarkable effectiveness both in induction of remissions and in maintenance of a disease free state.

During the past year, clinical trials of a number of drugs were continued or initiated. The list is a long one and included an epipodophyllotoxin (NSC 122,819), dibromomannitol (NSC 104,800), BCNU (NSC 79037), L-asparaginase (NSC 109,229), Poly IC (NSC 102,949), bleomycin (NSC 125,066), both imidazole carboxamides (NSC 45388 and NSC 82196), CCNU (NSC 79037), methyl CCNU (NSC 95441), acronine (NSC 403,169), etc. Several of these appear to be promising particularly bleomycin and methyl CCNU.

One of the most important developments during the past year, has been the

increased emphasis on the treatment of the major solid tumors. The same type of concerted effort which has been successful in acute lymphocytic leukemia, Hodgkin's Disease, and Burkitt's Lymphoma is being mounted against bronchogenic carcinoma, carcinoma of the colon, and carcinoma of the breast. Space and personnel limitations preclude the implementation of these programs in-house except for bronchogenic carcinoma. The NCI-VA Medical Oncology Unit has become one of the world's leading centers for the treatment of this tumor. A very productive program is now underway at the Mayo Clinic under contract for the evaluation of cytotoxic agents in carcinoma of the colon. During the year camptothecin (NSC 100,880), emetine (NSC 33669), streptozotocin (NSC 85998), CCNU (NSC 79037), and L-asparaginase (NSC 109,229) were studied. Streptozotocin (in pancreatic islet cell tumors) and CCNU showed a good deal of promise.

Unfortunately, efforts to develop a breast service at NIH has been quite fruitless to date, largely because of administrative problems concerning bed allocations in the Clinical Center. It is hoped that this will be resolved in the near future. Nevertheless, a program for drug trials in breast cancer will soon be implemented through the contract mechanism.

Based on the previous experience in the faster growing tumors there is good reason to be optimistic that important advances in the management of patients with breast, lung, and colon will soon be made.

Two years ago saw the establishment of the Human Tumor Cell Biology Branch which is concerned with fundamental studies of cellular control mechanisms and cell kinetics. This move represents recognition of a serious deficiency which is quite widespread in medical research. In general, there exists a large gulf between the so-called basic scientist and the clinician. There is usually a long delay before findings in laboratories utilizing animal, microbial, or cell systems are evaluated in man or in laboratory systems which readily can be related to man. The explanation for this deficiency lies in the fact that most basic scientists even in the cancer area do not consciously have as an objective the cure or control of neoplastic disease. The formation of this Branch within the clinical area was an attempt to remedy this deficiency. It also represented a significant broadening of program interests and recent events have demonstrated the wisdom of this decision. Unquestionably, without the existence of this resource, the rapid accumulation of information concerning tRNA, tRNA methylases, and the RNA dependent DNA polymerase in human tissue would not have occurred. Although the precise role of the polymerase in neoplasia remains to be defined,data now available suggest it may be important in neoplastic cell replication. Obviously, this has great implications for the treatment of cancer, particularly if potent and selective inhibitors can be identified.

The elucidation of the relationship of unusual tRNA's, elevated levels of tRNA methylase activity, and the RNA dependent DNA polymerase to neoplastic disease also may provide useful diagnostic techniques for early diagnosis and for the detection of residual disease after treatment. These may constitute biochemical markers which might be identified in urine,

blood, or tissues. It is not unreasonable to suggest that if such "markers" of residual or early disease were available the potential for cure or prevention of cancer would be greatly enhanced. Certainly, the remarkable reversal of mortality previously associated with female choriocarcinoma would not have been possible had it not been for the presence of urinary gonadotrophin to guide the therapist.

The developments and changes in Clinical Trials mentioned at the onset have resulted in a program which is probably one of the most comprehensive programs in basic, clinical and experimental cancer research to be found anywhere. This program developed through the years when the emphasis was primarily on the faster growing tumors but increasingly, major attention is being directed toward the common solid tumors. It is not overly optimistic to suggest that these efforts will be at least as successful as those in previous years.

SUMMARY REPORT

HUMAN TUMOR CELL BIOLOGY BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

This Branch is concerned with four areas of research: (1) cell cycle characteristics and cell kinetics; (2) physiological, cytogenetic, and biochemical control mechanisms in normal and neoplastic cells; (3) search for biochemical markers of minimal neoplastic disease; and (4) investigation of fundamental problems in marrow transplantation. Emphasis is placed on human studies and studies on fresh and frozen human tissue; tissue culture techniques and animal models are also utilized.

During the past year a number of exciting findings were reported by investigators from the Branch:

1. DNA Polymerases of Human Cells. During the year the Branch initiated an extensive investigation of DNA-dependent and RNA-dependent DNA polymerase activities in normal and leukemic human cells and in some solid tumors. This was prompted by: (a) the fact that the principal effect of many antitumor agents is through inhibition of DNA synthesis; (b) the finding in RNA oncogenic viruses of the first evidence for a "reverse transcriptase" (RNA-dependent DNA polymerase) which was virtually limited to the RNA tumor viruses and that this enzyme can be inhibited fairly selectively with some rifampicin derivatives.

Only one DNA polymerase (in E. coli) has been known for many years but last year a second DNA polymerase was discovered. Recently in this laboratory three DNA polymerases have been isolated and partially purified from human leukemic cells. One of these can catalyze DNA synthesis from an RNA template. This was the first finding of this type of enzyme in any animal cell. Although it remains to be proven whether this activity is the same as the enzyme from RNA oncogenic viruses, the template properties and inhibition by rifampicin derivatives (first described here) gave support to this possibility. If the enzyme is associated with an RNA oncogenic virus it is theoretically possible to prevent transformation with effective inhibitors. Unfortunately, an appropriate RNA template, i.e., RNA from a known oncogenic virus is not available. The polymerase work here and elsewhere has created a great deal of excitement because of the important implications for the control of neoplastic disease.

2. tRNA Methylation. Studies of tRNA methylating activities have shown that chemically (DENA) induced hepatomas in primates have increased tRNA

methylation and suggest that the appearance of new tRNA methylases. These changes occur prior to any histological evidence of neoplasia and are accompanied by the appearance of the serum protein, alpha fetoprotein. Similar findings (induction of methylases and alpha fetoprotein) also occur in the embryo, giving further support to the view that oncogenesis represents a form of gene derepression analogous to the fetal state.

An increase in the methylating enzymes was previously found in DNA oncogenic virus transformed cells (polyoma and SV40) compared to control cells (but not in RNA oncogenic virus transformed cells). Because of these findings the methylated bases of the tRNAs themselves were analyzed and an increase (3 to 4 fold) was found only in 3-methylcytidine in the transformed cells. An increase in methylated bases of the tRNA from human brain tumors had been reported earlier by other investigators. Stimulated by these findings a collaborative effort was undertaken with Dr. L. Levine of Brandeis University to develop antibodies against each methylated base of tRNA. This resulted in the first successful development of an immunologic approach to the detection of methylated purines and pyrimidines. The plan now is to attempt to increase the sensitivity of this method in an attempt to detect individual methylated bases in biological fluids, (e.g., cerebrospinal fluid and plasma) as potential biochemical markers for the presence of neoplastic disease.

3. Membrane Studies. It has recently been demonstrated that the membranes of transformed cells in culture are different from control cells, e.g., agglutination by concanavalin and in some systems an altered Km for glucose uptake. Because these findings might be immediately applicable clinically, normal and leukemic (acute and chronic) leukocytes from patients in remission and relapse were examined but no significant differences were found.

4. Low Molecular Weight RNA in Normal and Neoplastic Human Cells. In collaboration with Dr. Paul Gerber, comparisons of low molecular weight RNA species (4S and 5S RNA) were made using human lymphoblasts in tissue culture derived from normal donors, patients with infectious mononucleosis, Burkitt's lymphoma, PHA transformed lymphocytes, and from EB virus transformed lymphocytes. The patterns of RNA species in PHA transformed lymphocytes, "normal" lymphoblast cell lines, and infectious mononucleosis were similar. The RNA profiles of Burkitt lymphoma and EB virus transformed cells were similar to each other but differed considerably from the others.

5. RNA Uptake. Uptake of low molecular weight (4S) RNA by animal cells was demonstrated and conditions for increasing the penetration of these molecules more than 100 fold were developed. Uptake appears maximal in proliferating cells. With these conditions established, biological experiments are now in progress to study the effects of various RNA molecules from one cell on other cells.

6. Camptothecin. In collaboration with the Laboratory of Chemical Pharmacology the mechanism of action, anti-tumor effect, resistance, and cell cycle effects of the new anti-tumor agent, camptothecin, were studied. Inhibition of DNA synthesis and prevention of mitosis probably by binding to specific proteins appears to be the prime mechanism of action. Cross

resistance with other anti-tumor agents was not found.

7. Kinetic Studies, Spontaneous AKR Leukemia. Spontaneous AKR leukemia is being utilized as a model for human acute leukemia particularly for the study of cell cycle characteristics and the effects of cytotoxic agents. Utilizing cell separation techniques the cell cycle has been analyzed and the phases defined. This approach will permit a variety of studies including the effects of cytotoxic agents, biochemical events in the cell cycle, etc.

8. Cytogenetic Studies. Cytogenetic analysis in patients with acute myelocytic leukemia revealed that 70% had normal karyotypes, the remainder being aneuploid. There was a high incidence of G group abnormalities. In 6 patients with malignant melanoma, aneuploidy was found in the neoplastic cells of each individual.

9. Granulocyte Procurement and Transfusion. For the first time, data are being obtained in a controlled study demonstrating the value of granulocyte replacement in severely granulocytopenic individuals. It is becoming quite apparent as suspected, that to be of value, multiple granulocyte transfusions must be employed utilizing HL-A compatible donors.

10. Bone Marrow Transplantation. The marrow transplantation study in patients with acute leukemia has developed into a highly productive effort and successful "takes" have been achieved in a sizable group of recipients. Dog models are employed to resolve some of the problems encountered in man, particularly, graft-versus-host reactions (GVHR) which remain a serious complication in spite of histocompatibility as determined by HL-A matching and negative mixed leukocyte cultures. Hence, one of the problems under study in dogs is the modification of GVHR by post-transplant immunosuppression.

It is important to note that, although marrow transplantation has not achieved a cure, it has resulted in the induction of remissions in the patients in this study all of whom were considered refractory to conventional and experimental anti-leukemic agents.

Serial No. NCI-3409
1. Human Tumor Cell Biology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Leukocyte Kinetics and Characteristics of Cell Proliferation

Principal Investigators: Seymour Perry and Mitsuhiro Omine

Other Investigators: Harmon J. Eyre, Peter J. Rosen and Ada Brooks

Man Years:

Total: 3 1/2
Professional: 2 1/2
Other: 1

Objectives

1. To investigate the kinetic proliferation and cell cycle characteristics of leukemic blast cells in an attempt to thereby give a firmer theoretical grounding to the devising of more effective therapy.

2. To define the characteristics of the various phases of the cell cycle with emphasis on the resting cell (G_0).

3. To evaluate the function of granulocytes in the recipients of leukocyte transfusions.

Methods Employed

1. Leukemic or other cells from animals or man are separated, principally on the basis of size, in a special chamber containing a density gradient (sucrose, fetal calf serum, etc.). Separated cells are studied for DNA content, uptake of tritiated thymidine, and proliferative capacity as determined by transplantation and other techniques.

2. Peripheral leukocyte kinetics are studied with the use of $Na_2{}^{51}CrO_4$ and $DF^{32}P$ as labels in vitro. Following infusions, the specific radioactivity is monitored.

3. Surface scanning over organs in patients with a dual probe collimated scintillation probe is utilized to study the organ distribution of infused 51-chromate labeled cells.

Major Findings

1. Spontaneous AKR leukemia (mice) is considered a good model for human acute leukemia. Thymic lymphoblasts obtained from leukemic animals were separated as described above. The smaller cells were not in DNA synthesis and had a normal complement of DNA. The largest cells had twice the normal complement of DNA and the majority were in DNA synthesis. Between the extremes there were intermediate values of DNA content. However, all cells were capable of inducing leukemia in normal mice, indicating that if the small cells were in G_0, they were readily capable of recycling.

2. Utilization of the chamber separation technique has permitted an analysis of the cell cycle times of spontaneous AKR leukemia. Cell division occurs at approximately 18 hour intervals; T_s is 7 hours; G_1 + 1/2M, 9 hours; and G_2 + 1/2M, 2 hours. Examination of the per cent labeled mitoses curve confirms these estimates.

Proposed Course of Project

1. Work is in progress to elucidate the effects of various antitumor agents both in vivo and in vitro on spontaneous AKR leukemia. Particular emphasis is placed on agents which have been found to be effective in remission induction and maintenance.

2. These studies will soon be extended to patients with acute leukemia.

Honors and Awards

None

Publications

1. Perry, S., and Gallo, R. C.: Physiology of human leukemic leukocytes: Kinetic and biochemical considerations. In Gordon, A. S. (Ed.): Regulation of Hematopoiesis. New York, Appleton-Century-Crofts, 1970, pp. 1221-1272.

2. Perry, S.: The formation and destruction of leukocytes. In Greenwalt, T. J. and Jamieson, G. A. (Eds.): Formation and Destruction of Blood Cells. Philadelphia, J. B. Lippincott, 1970, pp. 194-206.

3. Rosen, P. J., Perry, S., and Schabel, F. M., Jr.: Proliferative capacity of leukemic cells in AKR leukemia. J. Nat. Cancer Inst. 45: 1169-1178, 1970.

4. Perry, S., and Wolff, S. M.: Neutropenia. In Conn, H. F. (Ed.): Current Therapy. Philadelphia, W. B. Saunders, 1971, pp. 223-224.

5. Perry, S.: The appraisal of myelopoiesis. Current Concepts. New Eng. J. Med. (in press).

Serial No. NCI-3414
1. Human Tumor Cell Biology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Cytogenetic Studies in Patients with Neoplastic, Hematologic, and Congenital Diseases and the Effect of Drugs and Viral Transformation upon Chromosomes of Cells Either in vivo or in vitro

Previous Serial Number: Same

Principal Investigator: Jacqueline Whang Peng

Other Investigators: Seymour Perry, Robert C. Gallo, Paul P. Carbone, Edward S. Henderson, C. J. Dawe, Paul Chretien, Berton Zbar, Paul Gerber, Richard Adamson, Robert G. Graw, Jr., Joost Oppenheim, Sanford Leikin, and P. M. L. Ming; Turid Knutsen and Elaine Lee

Cooperating Units: Medicine Branch; Surgery Branch; Laboratory of Pathology; Laboratory of Chemical Pharmacology; Biology Branch, NCI; Laboratory of Viral Immunology, DBS; Clinical Hematology Branch, NIAMD; Children's Hospital; Laboratory of Biochemistry; Dental Institute; Communicable Disease Center, Atlanta, and Pathology Department of Maryland University.

Man Years:

Total: 7 1/2
Professional: 5
Other: 2 1/2

Project Description:

Objectives

1. Cytogenetic studies in patients with leukemia or other malignancies.
2. The effect of therapeutic agents or drugs on human lymphocytes.
3. Studies of 1) the polyoma virus induced mouse salivary gland tumors, and 2) the chemically induced guinea pig leukemia and ascites tumors.
4. Blastoid cells in blast crisis patients with the blastic transformation of chronic myelocytic leukemia (CML).
5. Study of the differences in adult and newborn lymphocyte transformation by antigens and antigen-antibody complexes.

6. Study of the blood from pregnant women to evaluate the possibility of migration of fetal white cells through placenta.
7. To test marijuana extract distillate, O^8-THC and O-THC, DDT for possible embryotoxic effects and effects on chromosomes in the rabbit, Rhesus monkey, and human lymphocytes.
8. Evaluation of drugs (in vitro) which effectively inhibit RNA dependent DNA polymerase toward different kinds of leukemia.

Methods Employed

Standard cytogenetic techniques were used except as specified.

Major Findings

1. Chromosome studies were performed on primary or metastatic malignant melanoma from 6 patients all of them were aneuploid and had either hyperdiploid, near triploid, tetraploid or hypertetraploid cell lines. Three of the 6 patients had one or more marker chromosomes.

2. Karyotype of sand rat. The diploid number is 48, X chromosomes were the largest one and Y is a medium sized metacentric chromosome.

3. Cytogenetic studies on 103 AML patients showed 70.9% had a normal karyotype, 29.1% were aneuploid, no unique chromosome abnormalities were found, however, a significantly high incidence of G group involvement. Two percent of the so-called AML patients had positive Ph^1 chromosome. The so-called chromosome #10 secondary constriction (C-marker) was not found.

4. C-marker chromosome aberration is not a secondary constriction in human lymphocytes transformed by EB virus but a random gap or break that could have been caused by a non-specific viral infection.

5. L-asparaginase causes both resorption and malformation of the fetus (6.2-11.1%) but no chromosomal abnormalities were seen.

6. Epithelial origin of polyoma salivary tumors in mice was proved by chromosome marker cells.

Honors and Awards

None

Publications

1. Whang-Peng, J., Chretien, P., and Knutsen, T.: Polyploidy in malignant melanoma. Cancer 25: 1216-1223, 1970.

2. Ming, P. M. L., and Whang-Peng, J.: Cytogenetic studies of the somatic and germ-line cells in sand rats (Psammomys obesus). Diabetologia 6: 361-365, 1970.

3. Wepsic, H. T., Zbar, B., Whang-Peng, J., Borsos, T., and Rapp, H. J.: Guinea pig leukemia L_2C: Characterization of chromosomes and attempts to demonstrate antigenicity. J. Nat. Cancer Inst. 45: 99-105, 1970.

4. Whang-Peng, J., Henderson, E. S., Knutsen, T., Freireich, E. J., and Gart, J. J.: Cytogenetic studies in acute myelocytic leukemia with special emphasis on the occurrence of Ph^1 chromosome. Blood 36: 448-457, 1970.

5. Whang-Peng, J., Gerber, P., and Knutsen, T.: So-called C marker chromosome and Epstein-Barr virus. J. Nat. Cancer Inst. 45: 831-839, 1970.

Serial No. NCI-3415
1. Human Tumor Cell Biology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: NCI-IBM Blood Cell Separator and Leukocyte Transfusion Study

Previous Serial Number: Same

Principal Investigators: Robert G. Graw, Jr., Geoffrey P. Herzig, and Robert L. Carolla

Other Investigators: Seymour Perry, Shirley House, Carole Novick, Pamela Woodruff, Ruth Estabrook, William Z. Penland, Charles H. Kirkpatrick, Brigid G. Leventhal, Paul I. Terasaki, Alan J. Greenfield, G. Nicholas Rogentine, and Edward S. Henderson

Cooperating Units: Medicine Branch, NCI; Plasmapheresis Unit, NCI; Immunology Branch, NCI; Department of Surgery, Histocompatibility Typing Laboratory, UCLA School of Medicine; Histocompatibility Typing Laboratory, Microbiological Associates Contract; Program Analysis Branch, NCI; and Infectious Disease Section, NIAID

Man Years:

Total: 5
Professional: 3
Other: 2

Project Description:

Objectives

1. To continue development in the procurement of buffy coat collections in man utilizing the NCI-IBM continuous flow Blood Cell Separator and newer techniques.

2. To study the safety and efficacy of leukocyte replacement therapy in the supportive care of granulocytopenic patients.

3. To determine the kinetics of a successful leukocyte transfusion response.

4. To study the role of histcompatibility typing with regard to leukocyte transfusions.

Methods Employed

1. The operation of the NCI continuous flow Blood Cell Separator was continued with normal blood donors. Granulocytes were transfused into patients with documented bacterial septicemia, and local infections.

2. A new technique, filtration leukapheresis (Leukopak®) was examined in detail for the procurement of granulocytes in large quantities.

3. An extensive preclinical leukocyte transfusion program was initiated in dogs in order to study the effect of prophylactic granulocyte transfusions in conjunction with preclinical bone marrow transplantation studies. The use of hydroxyethyl starch and corticosteroids was also examined in these dog models.

4. In vitro studies (batch processing) of blood from normal donors was continued in conjunction with a contractor, ABCOR, in an attempt to improve leukocyte collection with available Cell Separator equipment.

5. Expanded computer techniques have been incorporated into the leukocyte procurement and leukocyte transfusion and histocompatibility evaluation programs.

6. The effect of in vitro x-irradiation on transfused blood products was investigated using phagocytic techniques and the mixed leukocyte culture.

Major Findings

1. Cell Separator collected granulocytes transfused from normal donors to patients with bacterial septicemia not having preformed antibody to the donor's cells have been demonstrated to be a significant adjunct to appropriate broad spectrum antibiotic treatment. One hundred percent of patients (10/10) receiving four or more consecutive granulocyte transfusions completely cleared all evidence of infection without any recurrence, while only 33% (9/27) patients in a matched control group survived with antibiotic treatment alone. The original results of posttransfusion granulocyte increments have been confirmed with a larger group of recipients. The transfusion of 10^{10} normal granulocytes to a recipient with a surface area of one meter square will produce a one-hour posttransfusion granulocyte increment of $650/mm^3$. The preliminary studies reported last year correlating HL-A typing and the absence of preformed leukocyte antibody with a reproducible transfusion response have been confirmed with continued transfusion experience.

2. Filtration leukapheresis in dogs and humans was found to be a satisfactory method to procure large quantities of normal granulocytes. In vitro

studies reflected almost normal bacteriocidal capacity of these cells, but on electron microscopy the cell membranes were severely distorted as was the nuclear architecture. Transfusion increments in humans with these Leukopak® processed granulocytes were consistently 25% (10 to 26%) of these increments obtained with Cell Separator cells. The consistently poor transfusion increments prompted leukokinetic studies in dogs. Intravascular survival ($DF^{32}P$) was markedly diminished (T 1/2 = 15 minutes, as compared to 6 hours for Cell Separator cells or whole blood), and autologous and homologous chromium-51 labeled transfused cells demonstrated abnormal localization in the spleen and liver.

3. Granulocyte transfusions were studied in a preclinical bone marrow transplantation model. Dogs prepared with 100 mg/kg of cyclophosphamide and given frozen autologous bone marrow uniformly died of gram negative septicemia (4/4). Only 1/8 animals similarly prepared, given prophylactic leukocyte transfusions during the period of maximal bone marrow hypoplasia died with gram negative septicemia and there were four long-term survivors. Leukapheresis, using the plasma expander hydroxyethyl starch, was generally unsuccessful because the dogs developed severe leukopenia during the second or third consecutive leukapheresis. Corticosteroids were demonstrated to elevate the circulating granulocyte pool and the number of granulocytes collected proportionately.

4. Batch processing of single units of fresh whole blood using standard plasmapheresis techniques confirmed that 40 to 50% of the total white blood cells and granulocytes could be collected with carefully selected centrifuge speeds. This contrasts to the 20% and 14% for the respective components collected using the Cell Separator. Mathematical models and in vitro experiments by ABCOR were aimed at determining the cause for this discrepancy. Prototype bowls designed to exploit these differences have been constructed and await testing in the preclinical studies.

5. Computer programs are now available to process and analyze leukocyte collection data. The initial leukocyte infusion data have been entered into a new infusion analysis file which has been created to better understand which clinical variables influence a successful leukocyte transfusion response. A newly created net histocompatibility ratio (NHR) concept has been programmed for computer comparison of recipient HL-A types and all prospective donors.

6. The effect of x-irradiation was examined on transfused blood products, since clinical graft-versus-host disease (GVHR) had resulted apparently from transfused CML granulocytes. The glucose monophosphate shunt activity and bacteriocidal assays of granulocyte function were not diminished at doses of x-irradiation to 10,000 R. The mixed leukocyte culture response (possible in vitro correlate of GVHR) was eradicated by 1500 R, but a diminished blastogenic response to phytohemagglutinin was preserved.

Proposed Course

1. Continued evaluation of better methods to procure granulocytes for transfusion (Cell Separator, Elutriation®, filtration leukapheresis). A special effort will be made to examine what deleterious effects transfused Leukopak® collected cells with shortened intravascular survival will have. An attempt will be made to alter the fiber component of the Leukopak® in order that less damaged cells can be collected.

2. Additional patients with proved bacterial septicemia will be given granulocyte transfusions, to confirm the observations made this year as to the effect of repetitive transfusions from compatible donors.

3. Continued evaluation of the prophylactic administration of granulocytes to patients following bone marrow transplantation.

4. Continued evaluation of leukocyte transfusions in infected dogs in order to determine the most effective leukocyte transfusion regimen.

5. Continued evaluation of prototype bowl designs in the dog leukapheresis studies.

6. Determine the significance of NHR analysis of HL-A typing data for selecting prospective WBC donors (in vivo and in vitro analyses).

7. Initiate a program of granulocyte typing for the selection of appropriate granulocyte donors.

Honors and Awards:

1. Graw, R. G., Goldstein, I., Eyre, H., and Terasaki, P. I.: Histocompatibility testing for leukocyte transfusion. Presented at the meeting of the American Association of Blood Banks, San Francisco, October 25, 1970.

2. Graw, R. G., Herzig, G. P., Eyre, H., Goldstein, I., Henderson, E. S., and Perry, S.: Treatment of gram negative septicemia in granulocytopenic patients with normal granulocyte transfusions. Presented at the meeting of the American Federation for Clinical Research, Atlantic City, May 1971.

Publications:

1. Eyre, H., Goldstein, I., Perry, S., and Graw, R. G., Jr.: WBC transfusion: Function of transfused granulocytes from donors with chronic myelocytic leukemia (CML). Blood 36: 432-442, 1970.

2. Goldstein, I., Eyre, H., Henderson, E., Terasaki, P., and Graw, R. G., Jr.: Prediction of leukocyte transfusion response by leukoagglutinin and lymphocytotoxicity testing. Transfusion 11: 19-24, 1971.

3. Graw, R. G., Jr., Goldstein, I., Eyre, H., and Terasaki, P.: Histocompatibility testing for leukocyte transfusion. Lancet 2: 77-78, 1970.

4. Graw, R. G., Jr., Buckner, C. D., Whang-Peng, J., Leventhal, B. G., Kruger, G., Berard, C., and Henderson, E. S.: Complication of bone marrow transplantation: Graft-versus-host disease resulting from supportive chronic myelogenous leukemia leukocyte transfusions. Lancet 2: 338-341, 1970.

5. Kirkpatrick, C. H., Rich, R., Graw, R. G., Jr., and Rogentine, G. N.: Treatment of chronic moniliasis with lymphocyte transfusions. Lancet (in press).

6. Siegel, S. E., Lunde, M. N., Gelderman, A. H., Halterman, R. H., Brown, J. A., Levine, A. S., and Graw, R. G., Jr.: Transmission of toxoplasmosis by leukocyte transfusions. Blood (in press).

7. Graw, R. G., Jr., Herzig, G. P., Eisel, R. J., and Perry, S.: Leukocyte and platelet collection with the continuous flow Blood Cell Separator from normal donors. Transfusion (in press).

8. Kirkpatrick, C. H., Rich, R. R., Graw, R. G., Jr., Smith, T. K., Michenberg, I. D., and Rogentine, G. N.: Treatment of chronic Mucocutaneous moniliasis by immunologic reconstitution. Clin. Exper. Immunol. (in press).

Serial No. NCI-3406
1. Human Tumor Cell Biology Branch
2. Section on Cellular Control Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Cell Regulatory Mechanisms in Normal and Neoplastic Cells and On New Approaches to Cancer Chemotherapy and the Development of Biochemical Markers of Neoplastic Cells.

Previous Serial Numbers: Same (in combination with NCI-3408 and NCI-3410)

Prinicipal Investigator: Robert C. Gallo

Other Investigators: Seitoku Fujioka, Robert Gallagher, R. Graham Smith, Paul Gerber, Richard Adamson, Jacqueline Whang-Peng, Flor Herrera, T. Phillip Waalkes, and Peter Wasserman

Cooperating Units: Laboratory of Viral Immunology, Division of Biologics Standards, and Laboratory of Chemical Pharmacology, NCI

Man Years:

Total: 9 3/4
Professional: 6 1/4
Other: 3 1/2

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

These objectives have primarily been pursued by the following approaches:

1. Characterization of tRNA species and tRNA methylation in normal adult and embryonic cells, growing and resting tissues,

and in various neoplastic cells. The rationale for this approach has been described in detail (J. Mol. Biol. 52: 195-219, 1970; Fourth International Miles Symposium on Molecular Biology: Biological Effects of Polynucleotides, Springer-Verlag, New York, in press).

2. Developing methods for getting specific RNA species into animal cells intact and to determine their biological effects.

3. Since the methylated bases of tRNA have been shown in some laboratories to be increased in some human tumors, an approach to quantitating various methylated bases by a sensitive and specific technique may be useful in quantitating residual tumor mass. An objective this year was to develop such an assay system. Similarly, in vitro studies of membrane properties of some neoplastic cells have indicated differences from normal cells (e.g., conconavalin agglutination and glucose transport). Therefore, one objective was to determine if human leukemic cells had these properties and if advantage could be taken of this to determine if the morphologically "normal" cells of remission leukemic patients are really normal.

4. Purification and properties of DNA polymerases (DNA-dependent and RNA-dependent) of human normal and leukemic cells and of RNA oncogenic viruses to determine ultimately:

   a. The function and number of DNA polymerases in these cells and viruses

   b. If RNA-dependent DNA polymerase is present in human leukemic cells and, if so, is it the same as the virion enzyme? If not, does it function in gene amplification during cellular differentiation? Is its activity altered in leukemogenesis? If found, is its activity necessary for maintenance of the neoplastic cell? Can it be used as a marker of neoplastic cells? If it is not necessary for maintenance of the neoplastic state will its continued inhibition prevent some relapses of animals or patients in remission, i.e., does the inciting agent in human leukemia (? virus) remain and produce subsequent transformations?

5. Development of potent and selective polymerase inhibitors to be evaluated on fresh human acute leukemic cells and proliferating normal lymphocytes in short term culture.

Methods Employed

1. The characterization of tRNA species (J. Mol. Biol. 52: 195-219, 1970), tRNA methylases (Cancer Res. 30: 2484-2492, 1970) have been previously described.

2. The isolation and quantitation of tRNA methylated bases from tissues were carried out by standard techniques.

3. The development of a sensitive technique for tRNA methylated bases was by an immunoassay developed by Dr. Lawrence Levine, Brandeis University (Biochemistry, in press).

4. Procedures for uptake of RNA into animal cells has been described (Proc. Natl. Acad. Sci. 67: 1943-1950, 1970). The demonstration of biological function of these RNA molecules added from one cell to another is evaluated in several systems.

5. Conconavalin agglutination tests were carried out as described by Sachs.

6. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Fourth International Miles Symposium on Molecular Biology: Biological Effects of Polynucleotides, Springer-Verlag, New York, in press; Blood 37: 282-292, 1971).

7. DNA polymerase activities were studied as reported (Nature 228: 927-929, 1970; Nucleic Acid-Protein Interactions and Nucleic Acid Synthesis in Viral Infection, Miami Winter Symposia 1971, D. W. Ribbons, J. F. Woessner, and J. Schultz, eds., North Holland Publishing Co., Amsterdam, in press).

8. Macromolecular synthesis, viability, mitosis in leukemic and normal cells, and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst., in press; Science 165: 400-402, 1969).

Major Findings

1. Previously we had examined tRNA species for all 20 amino acids in normal and leukemic cells and one major difference was found, tyrosyl-tRNA (J. Mol. Biol. 52: 195-219, 1970). This year the study was extended to compare a normal lymphoblast cell line with a plasma cell line derived from a patient with multiple myeloma and producing lambda chain (Bence-Jones) protein. Of four amino acids studied (aspartic acid, leucine, serine, and tyrosine) a significant difference was found only for tyrosine tRNA. This is the fourth system in which a finding of a tyrosyl-tRNA difference has been reported between a neoplastic and normal cell.

2. Studies of tRNA methylating activities have shown that chemically (DENA) induced hepatomas in primates have increases in tRNA methylation and suggest that new tRNA methylases appear. These changes occur prior to any histological evidence of neoplasia and are accompanied by the appearance of the serum protein, alpha fetoprotein. These exact same findings (induction of methylases and alpha fetoprotein) also occur in the embryo, giving further support to the view that oncogenesis represents a form of gene derepression analogous to the fetal state.

3. During the previous year an increase in the methylating enzymes was found in DNA oncogenic virus transformed cells (polyoma and SV-40) compared to control cells but not in RNA oncogenic virus transformed cells. Because of these findings the methylated bases of the tRNAs themselves were analyzed. A 3- to 4-fold increase in 3-methylcytidine was found in excess. An increase in methylated bases of the tRNA from human brain tumors had been reported earlier.

4. Because of the above findings a collaborative effort was made with Dr. L. Levine of Brandeis University to develop antibodies against each methylated base of tRNA. This was the first successful development of an immunologic approach to the detection of methylated purines and pyrimidines. The plan is to attempt to increase further the sensitivity of this method for a future approach to determining individual methylated bases in biological fluids (e.g., cerebrospinal fluid and plasma) as potential biochemical markers of tumor cells.

5. Studies were also carried out to determine if pre-neoplastic states could be detected by assaying the levels of tRNA methylases in skin fibroblasts from patients with various disorders associated with a high incidence of neoplasia. The results were negative. No correlation was found.

6. Recent exciting developments in in vitro systems have demonstrated that the membranes of transformed cells are different from control cells, e.g., agglutination by conconavalin and in some systems an altered Km for glucose uptake. Because these findings might be immediately clinically applicable (e.g., if so-called remission leukemic patients' leukocytes showed these subtle membrane changes, they might provide a basis for quantitation of morphologically non-recognizable but nevertheless neoplastic cells). Normal, leukemic (acute and chronic), remission and relapse leukocytes were examined. No correlations were found.

7. In collaboration with Dr. Paul Gerber comparisons of low molecular weight RNA species (4S and 5S RNA) were made between human lymphoblasts in tissue culture derived from normal donors, from patients with infectious mononulceosis, from Burkitt's lymphoma, from PHA transformed lymphocytes, and from EB virus transformed lymphocytes. With this system the range of lymphoblasts from normal non-tissue established, normal tissue culture established, non-neoplastic but proliferative disorders, and neoplastic proliferative disorder could be compared.

The patterns of the PHA transformed lymphocytes, "normal" lymphoblast cell lines, and infectious mononucleosis were similar. The RNA profiles of Burkitt lymphoma and EB virus transformed cells were similar to each other but differed considerably from the others.

8. Uptake of low molecular weight (4S) RNA by animal cells was shown and conditions for increasing the penetration of these molecules more than 100-fold was achieved. Uptake appears maximal in proliferating cells. With these conditions established, biological experiments are now in progress to study the effects of various RNA molecules from one cell on other cells.

9. In collaboration with the Pharmacology lab the mechanism of action, anti-tumor effect, resistance, and cell cycle effects of the new anti-tumor agent, camptothecin, were studied. Inhibition of DNA synthesis and prevention of mitosis probably by binding to specific proteins appears to be the prime mechanism of action. Cross resistance with other anti-tumor agents was not found.

10. DNA Polymerases of Human Cells. During the past year the Branch initiated an extensive investigation of DNA-dependent and RNA-dependent DNA polymerase activities in normal and leukemic human cells and in some solid tumors. This was prompted by (a) the fact that the main effect of many prominent anti-tumor agents is by inhibition of DNA synthesis; (b) the finding in RNA oncogenic viruses of the first evidence for a "reverse transcriptase" (RNA-dependent DNA polymerase) which was virtually limited to the RNA tumor viruses; combined with the knowledge that this enzyme can be inhibited fairly selectively with some rifampicin derivatives.

Findings. In E. coli only one DNA polymerase was known for many years. Last year a second DNA polymerase was discovered. Our laboratory has isolated and partially purified three DNA polymerases from human leukemic cells. One polymerase can catalyze DNA synthesis from an RNA template. This was the first finding of this type of enzyme in any animal cell. Although it remains to be proven whether this activity is the same as the enzyme from RNA oncogenic viruses, the template properties and inhibition by rifampicin derivatives (also first shown this year in our laboratory) gave support to this possibility. We were, however, handicapped from the start by not having the right RNA template, viz., RNA from a known human RNA oncogenic virus, to test with the leukemic enzyme. If it is from an RNA oncogenic virus it is theoretically possible to prevent transformation with effective inhibitors. Recent evidence in man also indicates that relapse may not only be due to failure to kill the last cell but also may involve additional transformations of normal cells to neoplastic cells. It may be possible to prevent this with these inhibitors. These concepts are presently under intensive investigation in a number of biochemical and biological systems.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on cellular control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Honors and Awards:

Dr. Gallo

1. Invited to present a summary on the Symposium on DNA Metabolism in Normal and Leukemic Cells. International Hematology Meeting, Munich, Germany. August 1970.

2. Invited to participate in the Symposium of the Fogarty International Center as Chairman of the Session on Alterations in tRNA and tRNA Methylases in Neoplasia. Presented a talk entitled "Transfer RNA and Transfer RNA Modification in Differentiation and Neoplasia."

3. Invited to present a lecture at the II Lepetit Colloquium on "The Biology of Oncogenic Viruses," Paris, France: "RNA- and DNA-Dependent DNA Polymerases of Human Normal and Leukemic Cells." December 1970.

4. Invited by the Department of Therapeutic Research of the University of Pennsylvania to lecture on "Transfer RNA and Growth and Differentiation of Normal and Neoplastic Cells." December 1970.

5. "Concepts on the Molecular Mechanisms of Leukemogenesis." Presented at the meetings of the American Society of Hematology in San Juan, Puerto Rico. December 1970.

6. Invited by the Department of Medicine, Oncology Division, of the Johns Hopkins University to present a talk "On the DNA Polymerases of Human Normal and Leukemic Cells." January 1971.

7. "Some Observations on DNA Polymerases of Human Normal and Leukemic Cells." Presented at the Third Annual Biochemistry-PCRI Winter Symposia in Miami, Florida. January 1971.

8. Invited by the Instituto Venezolano de Investigaciones Cientificas in Caracas, Venezuela to lecture on "Recent Observations on DNA Polymerase Activities of Normal and Leukemic Human Lymphoblasts," and "Studies on the Molecular Mechanisms of Leukemogenesis in Man." February 1971.

9. Invited by Mount Sinai Hospital in New York to lecture on "Recent Observations and Speculations on Human Leukemogenesis." February 1970.

10. Invited by the Leukemia Society of America, Inc., Western Pennsylvania Chapter, Pittsburgh to lecture on "Human Leukemia as an Aberration of Cell Differentiation." March 1971.

11. Invited by the Institute for Cancer Research of Columbia University in New York to lecture on "Observations on Human Leukemogenesis." March 1971.

12. Invited by the Connecticut Cancer Society of Waterbury, Connecticut to lecture on "New Approaches to Cancer Research and Possibilities for Practical Success." March 1971.

13. Invited by the American Cancer Society to participate in the Science Writers' Seminar in Phoenix, Arizona. Presented a talk entitled "Leukemia as an Aberration of Differentiation." April 1971.

14. Invited by the Department of Pharmacology of George Washington University to lecture on "The Finding of a Reverse Transcriptase (RNA-Dependent DNA Polymerase) in Human Acute Leukemic Cells." April 1971.

15. Invited by Cornell University to lecture on "Some Observations on DNA Polymerases of Human Lymphoblasts." April 1971.

16. Invited by M. D. Anderson Hospital and Tumor Institute of the University of Texas to lecture on "Some Biochemical Observations on Human Leukemogenesis." May 1971.

Publications:

1. Gallo, R. C., Yang, S. S., and Ting, R. C.: RNA-dependent DNA polymerase of human acute leukaemic cells. Nature 228: 927-929, 1970.

2. Herrera, F., Adamson, R. H., and Gallo, R. C.: Uptake of transfer ribonucleic acid by normal and leukemic cells. Proc. Natl. Acad. Sci. 67: 1943-1950, 1970.

3. Riddick, D. H., and Gallo, R. C.: The transfer RNA methylases of human lymphocytes: I. Induction by PHA in normal lymphocytes. Blood 37: 282-292, 1971.

4. Riddick, D. H., and Gallo, R. C.: The transfer RNA methylases of human lymphocytes: II. Delayed induction by PHA in lymphocytes from patients with chronic lymphocytic leukemia. Blood 37: 293-298, 1971.

5. Gallagher, R. E., Ting, R. C. Y., and Gallo, R. C.: Transfer RNA methylase alterations in polyoma transformed rat embryo culture cells. Proc. Soc. Exptl. Biol. Med. 136: 819-823, 1971.

6. Gallo, R. C., and Whang-Peng, J.: Observations on the regulatory effects of the transfer RNA minor base, N^6-Δ^2-isopentenyladenosine, on human lymphocytes. Fourth International Miles Symposium on Molecular Biology: Biological Effects of Polynucleotides. (Springer-Verlag, New York), in press.

7. Herrera, F., Adamson, R. H., and Gallo, R. C.: Transfer RNA as an informative molecule and its uptake by normal and leukemic human and murine leukocytes. In, NATO Symposium: Uptake of Informative Molecules by Living Cells. (Amsterdam: North Holland Publishing Co.), in press.

8. Gallo, R. C., Whang-Peng, J., and Adamson, R. H.: Anti-tumor activity, mechanism of action, and cell cycle effects of camptothecin. J. Natl. Cancer Inst., in press.

9. Fujioka, S., Ting, R. C., and Gallo, R. C.: Transfer RNA methylases of normal cells, virus transformed cells, and tumors derived from transformed cells. Cancer Res., in press.

10. Waalkes, T. P., Adamson, R. C., O'Gara, R. W., and Gallo, R. C.: Methylation of transfer RNA by monkey liver and hepatoma induced by N-nitrosodiethylamine. Archieves Internationales de Pharmacodynamie et de Therapie, in press.

11. Gallo, R. C.: Transfer RNA and transfer RNA methylation in growing and "resting" adult and embryonic tissues and in various oncogenic systems. Cancer Res., in press.

12. Gallo, R. C., and Adamson, R. H.: Asparagine, Asparaginase, and tRNA. Monographs on L-Asparaginase, in press.

13. Gallo, R. C., Yang, S. S., Smith, R. G., Herrera, F., Ting, R. C., Bobrow, S. N., Davis, C., and Fujioka, S.: RNA- and DNA-dependent DNA polymerases of human normal and leukemic cells. In, Proceedings of the II Lepetit Colloquium on The Biology of Oncogenic Viruses, L. G. Silvestri (ed.), (Amsterdam: North Holland Publishing Co.), in press.

14. Levine, L., Van Vunakis, H., and Gallo, R. C.: Serologic specificities of methylated base immune systems. Biochemistry, in press.

15. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., and Gallo, R. C.: Transfer RNA methylase activity in normal monkey liver and carcinogen induced hepatoma. Cancer Res., in press.

16. Gallo, R. C., and Whang-Peng, J.: Dibutyryl cyclic 3',5 AMP enhances transformation of human immunocompetent cells. J. Natl. Cancer Inst., in press.

17. Fujioka, S., and Gallo, R. C.: Aminoacyl transfer RNA profiles in human myeloma cells. Blood, in press.

18. Gallo, R. C., Yang, S. S., Smith, R. G., Herrera, F., Ting, R. C., and Fujioka, S.: Some observations on DNA polymerases of human normal and leukemic cells. In, Nucleic Acid-Protein Interactions and Nucleic Acid Synthesis in Viral Infection, Miami Winter Symposia 1971, D. W. Ribbons, J. F. Woessner, and J. Schultz (eds.), (Amsterdam: North Holland Publishing Co.), in press.

19. Gallo, R. C.: Synthesis and metabolism of DNA and DNA precursors by human normal and leukemic leukocytes: A summary of recent information. Acta Hematologica, in press.

Serial No. NCI-3417
1. Human Tumor Cell Biology Branch
2. Section on Cellular Control Mechanisms
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical and Pharmacological Studies Designed to Improve Clinical Cancer Chemotherapy: A) Mechanism of Action of Select Tumor Agents; BI) Biochemical Markers of Malignancy; BII) (α-Fetal Protein and Monkeys with Hepatoma Induced by N-nitroso Dethylamine;: Clinical Correlations); C) Clinical Pharmacology of Selected Drugs; d) Enzymatic Reactions which may be Exploited for the Development of Better Chemotherapeutic Agents.

Previous Serial Number: Same

Principal Investigators: T. Phillip Waalkes, Robert C. Gallo, Richard Adamson, Vincent T. DeVita, and Flor de Maria Herrera.

Other Investigators: R. Graham Smith, Peter Wasserman, Charles Vogel, and Michael Walker.

Cooperating Units: Laboratory of Chemical Pharmacology and Experimental Therapeutics, NCI; Solid Tumor Service, Medicine Branch, NCI, and Baltimore Cancer Research Center, NCI.

Man Years:

Total: 3
Professional: 2
Other: 1

Project Description:

Objectives

A) To obtain information regarding the biochemical and pharmacologic mechanisms whereby chemotherapeutic agents, known to be at least partially effective against various malignancies in man, appear to act. It is considered that information of this type will be helpful not only in the selection of possible analogs, the synthesis of specific compounds, and a rational approach to combination therapy, but also in the understanding of the basic biochemical processes of malignancy and malignant cell growth. The current approach involves the study of tRNA and its methyla-

tion in specific neoplastic systems. Various reports have confirmed the finding that hypermethylation of tRNA is a characteristic of the tumor cell. Although the function served by the addition of methyl groups to the nucleic acid bases of tRNA is not clear, the important role of tRNA in protein synthesis lends further emphasis to the possibilities.

BI) Because of the obvious need for better methods to assess the status of patients with cancer, to evaluate response to therapy and the course of disease, efforts are in progress to determine directly related biochemical parameters which may lend themselves to potential use.

C) I. Pentamidine. Pentamidine has been found to be a useful therapeutic agent for diffuse interstitial pneumonia caused by Pneumocystis carinii. This serious complication is not uncommon in patients with malignancy receiving immunosuppressive agents. Frequently, administration of pentamidine to seriously ill patients is associated with rising BUN and evidence of renal toxicity.

II. Imidazole mustard (NSC-82196). [Imidazole-4 (or 5)-carboxamide, 5 (or 4)-[3,3-Bis (2-chloroethyl)-1-triazeno]]. The compound has proven to be active in the CCNSC screening system.

D) Specific enzyme reactions involving RNA and DNA are being studied from a variety of approaches (see Dr. R. C. Gallo's annual report). In particular, RNA dependant DNA polymerase and DNA dependant DNA polymerase in several different tissues and types of cells are being evaluated as to their relationship to tumor cell (e.g., acute leukemia) growth and proliferation. Where unique features exist or where specific differences are found which may be therapeutically exploited, careful evaluation will be made to determine the best methods and means of approach.

Methods Employed

A) The L1210 system both in vitro (tissue culture) and in vivo was used to evaluate the effects of specific chemotherapeutic agents on the methylation of tRNA. The agents studied to date include methotrexate, vincristine, BCNU, Poly IC, 6-MP, and MIH. In vitro,methotrexate has a definite inhibitory effect on methylation at 24 to 48 hours. None of the other compounds showed a similar effect. Further studies indicate that methotrexate also causes a profound inhibition on protein synthesis in the same system under similar conditions. This effect may well be the explanation for the changes found in the decreased methylation of tRNA.

BI) Within tumor cells tRNA is hypermethylated. Consequently, because brain tumors, compared to normal frontal lobe, have a much higher proportion of methylated nucleic bases as found by Viale, studies have been made to

determine whether in spinal fluid from patients with brain malignancies evidence of increased methylation of tRNA exists. To date, compared to spinal fluid from normal controls, there is no indication of elevated levels for tRNA methylation enzymes. However, the possibility remains that nucleic acid methylated bases may be present in increased amounts in spinal fluid or blood or urine of such patients. N^2-methyl guanines specifically have been found to be present in proportionally large amounts in various types of tumors. By fluorometric techniques and assay, after proper development and the acquisition of necessary instrumentation, investigations are planned to study the level of selected methylated bases in spinal fluid, blood and urine. Application of the methods, if appropriate, can be made for patients with types of malignancy other than brain tumors.

In addition, by means of the following contracts currently under consideration the possibility of developing methods to detect methylated nucleic acids in body fluids is being investigated.

1) Liquid chromatographic separations - Oak Ridge National Laboratories, Dr. Charles Scott - using high resolution, high pressure commercial type columns with a variety of ways for identification of individual components in body fluids.

2) Gas chromatography - contractor not selected.

3) Radioactive immunoassay techniques - Brandeis University - Dr. Levine - previous work has indicated the feasibility to prepare antibodies to selected methyl bases. Efforts will be made to increase the sensitivity of these methods by radio-immuno-assay.

Initially, after the techniques have been developed, evaluation of normal ranges will be made. Then a survey using body fluids (particularly urine) from cancer patients will be made.

BII) Recent reports show that specific malignancies in humans (i.e., hepatomas, etc.) frequently are associated with the presence of an α-fetal protein. A similar situation exists in monkeys harboring a hepatoma induced by the simple chemical N-nitroso diethylamine. Of interest, the α-fetal protein appears prior to any histologic evidence of a liver tumor. Consequently, the α-fetal protein is indicative of a pre-malignant state. Of further interest, studies of the methylation of tRNA show hypermethylation compared to normal liver, not only with extracts of hepatoma tissue but also with "normal" liver of monkeys with a positive test for α-fetal protein. Further exploration of these biochemical differences is in progress. Possibilities of correlations with patients with hepatoma, etc., are considered.

In addition, a natural inhibitor of tRNA methylase activity is present in normal liver extracts but absent in fetal or new born liver and hepatoma. Further studies are contemplated in an effort to determine the nature and activity of these inhibitors and to evaluate their presence or absence in body fluids or tissues of patients with malignancy.

CI) Previously, clinical pharmacologic studies have been hampered by the lack of sufficiently sensitive methods of assay for pentamidine in blood and urine. A sensitive and specific method was developed and studies in patients carried out. After intramuscular administration once a day of the drug, plasma levels were found to be very low with no significant peak. Highest levels were noted in patients with elevated BUN. Urinary excretion was prolonged for several weeks after a 10-day course of treatment. Studies in mice after intraperitoneal injection showed that pentamidine is stored in the tissues for prolonged periods with the highest level in the kidneys and then the liver. Excretion is predominantly by the urinary tract with a small percent by way of the biliary tree. Pentamidine appears to be stored in tissues and excreted over a period of several weeks largely as intact compound.

Further studies are contemplated to determine the site and chemical nature of the attachment of pentamidine in renal tissue and to determine methods by which kidney toxicity and deposition can be altered by increasing urinary excretion by the drug.

CII) Studies have been carried out to determine the physiological disposition, absorption, excretion, and metabolism in mice and dogs, after oral and parenteral administration of the C^{14} labeled compound. These studies were hambered by the sensitivity of the compound to light and its instability in solution. The results of these investigations have been reported. (No further studies contemplated on my part.)

D) (See Dr. R. C. Gallo's annual report.) Participating in studies which involve specific tissues and cells including RNA viruses (e.g., Rauscher murine leukemia virus), human lymphocytes grown in culture (#1788), PHA stimulated normal lymphocytes and acute leukemia WBC. Enzymes are being purified, defined and characterized. Antibody and inhibitor studies are being carried out on RNA dependant DNA polymerase and on DNA dependant DNA polymerase.

Honors and Awards:

None

Publications:

BII) 1. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., and Gallo, R. C.: Methylation of tRNA by monkey liver and hepatoma induced by N-nitrosodiethylamine. Arch. Int. Pharmadocynamie et Therap.,(in press).

2. Waalkes, T. P., Adamson, R. H., O'Gara, R. W., and Gallo, R. C.: tRNA methylase activity in normal monkey liver and carcinogen induced hepatoma. Cancer Res.,(in press).

CI) 1. Waalkes, T. P., and DeVita, V. T.: The determination of pentamidine (4,4[1]-diamidinophenoxypentane) in plasma, urine and tissue. J. Lab. Clin. Med. 75: 871-878, 1970.

2. Waalkes, T. P., Denham, C., and DeVita, V. T.: Pentamidine: Clinical pharmacologic correlations in man and mice. Clin. Pharmacol. Therap. 11: 505-512, 1970.

CII) 1. Vogel, C., Denham, C., Waalkes, T. P., and DeVita, V. T.: The physiological disposition of the carcinostatic Imidazole-4 (or 5)-carboxamide, 5 (or 4)-[3,3-bis (2-chloroethyl)-1-triazeno]. Cancer Res. 30: 1651-1657, 1970.

Serial No. NCI-6300
1. Human Tumor Cell Biology Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Title: Bone Marrow Transplantation

Previous Serial Number: None

Principal Investigators: Robert G. Graw, Jr., Geoffrey P. Herzig, and Robert L. Carolla

Other Investigators: Edward S. Henderson, Harvey Gralnick, Costan Berard, Ronald A. Yankee, G. Nicholas Rogentine, Brigid G. Leventhal, John Northup, John Bacher, Roger Halterman, Joan Bull, Regina Dowling, Gary Peck, Carole Novick, Shirley House, Christopher Merritt, Gerhard Krueger, Jacqueline Whang-Peng, and Ralph Johnson

Cooperating Units: Medicine Branch, NCI; Division of Research Services, NIH; Clinical Pathology Deparment, CC; Plasmapheresis Unit, NCI; Immunology Branch, NCI; Hematology Service, CP; Dermatology Branch, NCI; Radiation Branch, NCI, and Armed Forces Radiobiologic Institute, National Naval Medical Center

Man Years:

Total: 7
Professional: 4
Other: 3

Project Description:

Objectives

1. To evaluate the safety and efficacy of autologous and allogeneic bone marrow transplantation in humans.

2. To study the antileukemic effect of allogeneic bone marrow transplantation.

3. To examine the role of HL-A matching for the selection of compatible bone marrow donors.

4. To study the graft-versus-host reaction as influenced by matching of donors and recipients for transplantation antigens; transfusion of supportive blood products; use of immunosuppressive agents; and modification of the host's immune defense system.

5. To evaluate what role frozen autologous bone marrow can play in the management of patients with malignancy.

Methods Employed

1. Clinical trials of allogeneic bone marrow transplants from siblings matched for HL-A antigens to patients with acute leukemia refractory to conventional chemotherapy were continued.

2. Autologous bone marrow storage in liquid nitrogen was initiated on patients with acute leukemia in remission status.

3. Preclinical evaluation of frozen autologous bone marrow was made in dogs and monkeys.

4. A preclinical allogeneic bone marrow transplantation program was initiated in dogs to examine in greater depth which factors influence graft acceptance, graft rejection, and the graft-versus-host reaction.

Major Findings

1. Eleven HL-A antigen and mixed leukocyte culture (MLC) matched allogeneic bone marrow transplants were attempted in nine multiply transfused patients with acute leukemia in an effort to evaluate a possible antileukemic effect and toxicity to the recipient of the immunosuppressive regimens and marrow engraftment.

   All but one patient were considered to be refractory to conventional chemotherapy. Eight patients were engrafted as indicated by a change to donor red blood cell type, karyotype, immunoglobulin allotype, speed of marrow repopulation of bone marrow, and/or the occurrence of the graft-versus-host reaction (GVHR). The median duration of remission in the patients successfully engrafted was 67 (range 10 to 132) days and the median survival of all patients following preparation for marrow transplantation was 90 (range 20 to 519+) days.

   Two forms of pretransplant immunosuppression were employed: 1) a four-day cytoxan regimen modified from Santos and 2) total body gamma irradiation (950 to 100 rads). All patients had received numerous random donor blood product transfusions (12 to 157 units) prior to attempting bone marrow transplantation.

   In one case platelets from the HL-A matched bone marrow donor had been given during the pretransplant period. None of these transfused blood

products appeared to jeopardize graft acceptance, even though several recipients had circulating antibody to leukocyte antigens contained in random donor blood products. One patient (red blood cell type O Rh negative) failed to engraft on two occasions from her HL-A matched donor (red blood cell type A Rh positive).

Two of four patients who developed clinical GVHR which was confirmed by histopathologic criteria died with gram negative septicemia during fulminant GVHR. One of these patients received unirradiated leukocyte and platelet transfusions from the HL-A matched bone marrow donor during the posttransplant period which may have influenced the magnitude of GVHR. The other patient was one of two who received no posttransplant immunosuppression with methotrexate.

Three patients developed cytomegalovirus (CMV) infection during the posttransplant period. Please see the Medicine Branch, NCI, annual report for additional details concerning the above clinical trials. This large series of HL-A matched bone marrow transplants emphasized the need for additional preclinical studies in marrow transplantation, particularly aimed at answering questions concerned with the role of posttransplant immunosuppression in modifying GVHR; the true incidence and severity of GVHR in mixed leukocyte culture compatible (HL-A matched) situations; the influence of x-irradiated and unirradiated supportive transfusions on GVHR; the role of pretransplant sensitization to donor transplantation antigens on graft acceptance; better control of infection during the posttransplant period; and finally what role a controlled GVHR can play in an immunotherapeutic approach to malignancy. The antitumor effect obtained in these patients to date has been minimal except in those patients with GVHR where death usually occurred too early (31 and 69 days) to evaluate the effect of GVHR on residual tumor. The patient population used for these preliminary studies was chosen because of the unknown risks involved with this new procedure and not as the ideal experimental setting to evaluate the antileukemic immunoadjuvant effect of allogeneic marrow transplantation. The ideal patient to test this hypothesis should be the new leukemic patient who has the least amount of residual tumor following an intensive course of chemotherapy. However, this patient can be studied only when the full risks of marrow transplantation can be known and predicted for any individual patient based on available donors, histocompatibility antigen matching, and complete knowledge of the risks of the procedure itself.

2. Autologous bone marrow has been frozen from fine patients with acute leukemia in remission in order that they could be given larger doses of chemotherapy (i.e., the four-day cytoxan regimen used for allogeneic bone marrow transplantation) when their leukemia recurs. This group of patients can become the appropriate control group for the previously described marroe transplants in evaluating whether the immunosuppressive regimen alone or in combination with allogeneic marrow is responsible for the antileukemic effect observed. This frozen marrow can also be

used for "bone marrow rescue" experiments in patients developing GVHR after receiving allogeneic marrow transplants.

3. Methods of freezing bone marrow were examined and undertaken in animals before proceeding with the human marrow storage program. Bone marrow has been frozen and transfused after lethal doses of cytoxan or x-ray to 50 dogs and 6 monkeys. Results indicate conclusively that the frozen nucleated marrow cells contain sufficient numbers of viable "stem cells" on thawing to repopulate an animal following lethal bone marrow ablation. Techniques were developed in our laboratory for percutaneous marrow aspiration from humeri and femurs which give sufficient numbers of cells for autologous and/or allogeneic marrow transplantation in dogs without sacrificing the donor animals. The animals prepared with x-ray or cytoxan provide excellent models for the study of leukocyte transfusions alone or in combination with marrow transplantation (see project No. NCI-3415).

4. In order to study what factors influence or modify the graft-versus-host reaction, allogeneic marrow transplants were initiated in dogs.

   Dogs given 950 to 1000 R total body x-irradiation with marrow transplants survived 7 to 10 days, finally succumbing to gram negative bacterial septicemia. Dogs given allogeneic marrow following 1000 R die on day 4 or 5 with fulminant GVHR and septicemia. The time to death and/or management of the animal with GVHR is not altered by the administration of methotrexate posttransplant as has been reported to be effective by the Seattle Transplant Group. This finding, if confirmed, with continued experiments, may have significance for the clinical program since our main basis for using posttransplant immunosuppression has been based on the results of canine experiments by the Seattle Group.

Preliminary attempts were made to fractionate dog bone marrow on ficoll gradients in order to remove a mature immunocompetent fraction possibly responsible for GVHR. These experiments were successful in that morphologically distinct populations of cells could be separated. However, this work was not continued after the workshop held in Holland during the summer of 1970, since experimental evidence in primates and early clinical trials of this technique indicated that only a delay in the onset of GVHR was observed and that fractionation per se did not significantly alter the incidence of GVHR.

Litters of English-American foxhounds have been examined for mixed leukocyte culture compatible siblings in order that marrow transplants can be performed in a model similar to that available in humans matched for HL-A antigens. These experiments will determine the frequency and severity of GVHR in matched siblings as opposed to unmatched and provide information as to what effect transfusion support from matched and mismatched donors will have on GVHR.

Using these same families of foxhounds, experiments have been undertaken to study the effect of enhancing antibody on graft acceptance and GVHR. Enhancing antibody may provide a tool to allow marrow grafts between mismatched individuals, and these preclinical models are required to examine its usefulness. Additional experiments are in progress to study the effect of in vitro incubation of allogeneic bone marrow with antibodies to dog immunoglobulins as a method of preventing GVHR by destroying the recognition cell component for GVHR in vitro before transfusing the marrow cells to the host.

Since murine tumor systems have demonstrated a correlation of antitumor activity with the amount of GVHR, we have established a model system to study "marrow rescue" from GVHR. This is accomplished by destroying the allogeneic graft causing GVHR and associated antitumor effect and replacing this foreign marrow with stored autologous or isogeneic marrow. If sufficient parameters of safety can be established in these experiments, this type of experiment can soon be tried in the clinical setting.

Proposed Course

1. Continue HL-A matched marrow transplants in patients with malignancy or aplastic anemia refractory to standard therapy.

2. Continue autologous bone marrow grafting experiments in patients with malignancy.

3. Continue preclinical evaluation of allogeneic marrow grafting, particularly focusing on factors influencing GVHR, graft acceptance, and antitumor activity. Experiments currently in progress or planned include the following:

    a. Evaluation of posttransplant immunosuppression with methotrexate, cytoxan and antilymphocyte serum.

    b. Continue grafting experiments in mixed leukocyte culture compatible canine siblings.

    c. Evaluation of enhancing antibody on GVHR.

    d. Evaluation of antibodies to dog immunoglobulins on GVHR.

    e. Continue "marrow rescue" for control of GVHR.

    f. Evaluation of in vitro inhibitors of the mixed leukocyte culture response on GVHR (amantadine HCl, thymidine suicide experiments).

    g. Evaluation of antitumor effects of allogeneic marrow grafting in spontaneous occurring canine lymphomas.

Honors and Awards:

Robert G. Graw, Jr.

1. Invited to present lecture at the Johns Hopkins University School of Medicine, Department of Medicine, June 9, 1970. Title: Bone Marrow Transplantation in Patients with Acute Leukemia.

2. Invited to present lecture at the Twelfth Annual Science Writers Seminar sponsored by the American Cancer Society, March 27, 1970, San Antonio, Texas. Title: Granulocyte Procurement and Transfusion and Bone Marrow Transplantation in Patients with Acute Leukemia.

3. Graw, R. G., Leventhal, B. G., Yankee, R. A., Rogentine, G. N., Whang-Peng, J., Halterman, R., Herzig, G., and Henderson, E. S.: Allogeneic Bone Marrow Transplantation for Patients with Acute Leukemia. Presented at the Third International Congress of Transplantation, Hague, The Netherlands, September 1970.

4. Graw, R. G., Jr., Yankee, R. A., Leventhal, B. G., Rogentine, G. N., Herzig, G. P., Halterman, R. H., Krueger, G., Berard, C. W., and Henderson, E. S.: Graft-versus-host Disease Complicating HL-A Matched Bone Marrow Transplantation. Presented at the Winter Bone Marrow Conference, Pittsburgh, Pennsylvania, November 13, 1970.

5. Graw, R. G., Jr., Leventhal, B. G., Yankee, R. A., Rogentine, G. N., Whang-Peng, J., Herzig, G. P., Halterman, R. H., Berard, C., Krueger, G., and Henderson, E. S.: Spectrum of the Graft-versus-host Reaction Complicating HL-A Matched Allogeneic Bone Marrow Transplantation. Presented at the meeting of the American Society of Hematology, San Juan, Puerto Rico, December 1970.

Publications:

1. Graw, R. G., Jr., Brown, J. A., Yankee, R. A., Widerlite, L. W., Leventhal, B. G., Whang-Peng, J., Rogentine, G. N., and Henderson, E.S.: Transplantation of HL-A and MLC identical bone marrow to patients with acute lymphocytic leukemia. Exper. Hemat. 20: 35- , 1970.

2. Graw, R. G., Jr., Brown, J. A., Yankee, R. A., Leventhal, B. G., Whang-Peng, J., Rogentine, G. N., and Henderson, E. S.: Transplantation of HL-A identical bone marrow to a patient with acute lymphocytic leukemia. Blood 36: 736-747, 1970.

3. Graw, R. G., Jr., Buckner, C. D., Whang-Peng, J., Leventhal, B. G., Krueger, G., Berard, C., and Henderson, E. S.: Complication of bone marrow transplantation: Graft-versus-host disease resulting from supportive chronic myelogenous leukemia leukocyte transfusions. Lancet 2: 338-341, 1970.

4. Graw, R. G., Jr., Rogentine, G. N., Yankee, R. A., Leventhal, B. G., Whang-Peng, J., Herzig, G. P., Halterman, R. H., Krueger, G., Berard, C., and Henderson, E. S.: Graft-versus-host reaction complicating HL-A matched bone marrow transplantation. Lancet 2: 1053-1055, 1970.

5. Krueger, G. R. F., Berard, C., Elias, P. M., Graw, R. G., Jr., Rogentine, G. N., Leventhal, B. G., Yankee, R. A., Herzig, G. P., Halterman, R. H., and Henderson, E. S.: Morphology of graft-versus-host reaction in HL-A matched bone marrow transplantation and its differential diagnosis. Am. J. Path. 63: 179-202, 1971.

6. Graw, R. G., Jr., and Santos, G. W.: Bone marrow transplantation in patients with leukemia. Incidence and severity of graft-versus-host disease in successful HL-A matched transplants. Transplantation 11: 197-199, 1971.

7. Graw, R. G., Jr., Leventhal, B. G., Yankee, R. A., Rogentine, G. N., Whang-Peng, J., McGinniss, M., Herzig, G. P., Halterman, R. H., and Henderson, E. S.: HL-A and mixed leukocyte culture matched allogeneic bone marrow transplantation in patients with acute leukemia. Transplantation Proceedings (in press).

8. Graw, R. G., Jr., Yankee, R. A., Leventhal, B. G., Rogentine, G. N., Herzig, G. P., Halterman, R. H., Krueger, G., Berard, C. W., and Henderson, E. S.: Graft-versus-host disease complicating HL-A matched bone marrow transplantation. Experimental Hemat. (in press).

9. Halterman, R. H., Leventhal, B. G., Graw, R. G., Yankee, R. A., Rogentine, G. N., Herzig, G. P., and Henderson, E. S.: Immunocompetence of patients following HL-A matched bone marrow transplantation. Exper. Hemat. (in press).

SUMMARY REPORT

MEDICINE BRANCH

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

The intramural program of the Medicine Branch can be divided into four major categories identified in the National Cancer Institute, Chemotherapy Program. These are:

1. The initial clinical trials of antitumor agents in man.
2. Supportive care of patients receiving antitumor therapy.
3. Delineation of new therapeutic agents in rapidly and slow-growing tumors.
4. Tumor eradication.

The program focuses on specific diseases such as malignant lymphoma, acute leukemia and bronchogenic carcinoma. In addition, during the past year studies have begun in patients with breast cancer and ovarian carcinoma. The program in Africa at Makerere has continued to investigate Burkitt's tumor, melanoma, hepatoma and Kaposi's sarcoma. An available in-patient population of 116 beds exists in the Medicine Branch and in Kampala and our out-patient population has increased to about 500 patients. Laboratory programs are closely allied to the clinical studies and most investigators in the Medicine Branch have responsibility for clinical research programs in addition to laboratory projects. Laboratory projects include pharmacology, cell kinetics, immunology, gnotobiology, and data processing. All of our units have individuals skilled in pharmacology and cell kinetics. Recently we have developed a central data processing and operations office. We have expanded our Laminar Air Flow beds from two to six and have developed contract facilities to help carry on bacteriological surveillance.

In Fiscal Year 1971, four more phase I drug studies were completed. These included bleomycin (NSC 125066), Poly I-C (NSC 120949), imidazole carboxamide dimethyltriazeno bis-chloroethyl (NSC 82196), and camptothecin (NSC 100880). Three new drugs were begun on phase I clinical trials. These are methyl CCNU (NSC 79037), isophosphamide (NSC 109742), ICRF-159 [bis(3-5 dioxopiperazine)]. Thus, these drugs have been made available to our own disease-oriented program as well as to other groups such as the Mayo Clinic, the Eastern Cooperative Oncology and Leukemia Group B Groups.

1. Pharmacologic Studies

Evaluation of new agents involves the understanding of the physiologic disposition of the agent and excretion. During the past year the Medicine Branch completed studies of asparaginase, camptothecin and bleomycin. Pharmacologic studies are anticipated for isophosphamide, methyl CCNU and ICRF-159.

2. Supportive Care

The Medicine Branch has as one of its main goals the development of protected environments for patients receiving intensive chemotherapy. Portable laminar air flow units have been expanded from one in October 1969, to six in March 1971, including two at the Washington Veterans Administration Hospital. These units combine laminar flow and high efficiency filtration and provide a more convenient isolation area for patients. The numbers of organisms in the air and on the surface have been markedly decreased. Patients are randomly allocated to Group I, who receive antibacterial baths, sprays, topical antisepsis, and oral antibiosis and a low bacterial diet and are confined to the Laminar Air Flow Isolator. Group II patients are given the same oral, nonabsorbable antibiotics as patients in Regimen I but otherwise treated conventionally; in Group III the patients are treated in the conventional manner except with no antimicrobial prophylaxis.

Recent evaluation of these techniques has indicated a dramatic and statistically significant decrease in the severe pseudomonas infections, 27% of Group III patients to 6% and 0% of Group II and I, respectively. Of great importance was the fact that no infectious deaths occurred during induction chemotherapy in Group I patients out of 16 consecutive trials. Twenty-two percent of Group II and 27% of Group III patients died of severe infection as the primary cause of mortality. Most infectious deaths in Group III were caused by pseudomonas while the deaths in Group II occurred with a variety of other organisms other than pseudomonas. It was also important to note that the oral antibiotics did not increase the incidence of fungal infections. Infectious complications were less frequent at all granulocyte levels; the patients in Group I and II experiencing one-third as many days with severe infection as patients in Group III at the same granulocyte level. Moreover, the time to onset of first infection was prolonged significantly for Groups I and II. This would mean that the oral antibiotics and the isolator allow sufficient time to administer adequate chemotherapy. While the results of induction chemotherapy indicated no difference among the three groups, there was a markedly better survival for the patients in the protected environment. Twelve out of 15 patients survived for more than 100 days, 25 of 35 in Group II, and 13 of 24 in Group III.

This controlled randomized study of protected environments will continue until we can demonstrate with statistical significance the ultimate utility of these techniques. It should be possible to reach this goal within the next 12 months. The activities of patient protection research will be expanded with the addition of a Ph.D. microbiologist who will assist the Medical Officer and aid in the development techniques for opportunistic pathogens, including using new anti-microbial and other techniques against herpes virus and fungi.

3. Marrow Transplantation

An active program in bone marrow transplantation has been in progress for the last year. A total amount of 11 bone marrow grafts in patients with acute leukemia have been attempted using the technique of four-day cyclophosphamide at 45 mg/day for immunosuppression and marrow ablation. These studies have indicated that in spite of HLA genetic identity and leukocyte culture reactivity, a fatal graft-versus-host reaction can occur. This has occurred in a total of 10 of the patients including deaths. It is obvious that post-transplant immunosuppression will be necessary. We have also learned that marrow grafts can be successfully performed in patients who have received prior blood products. Therefore, we find that successful grafting can be deferred until drug trials with known experimental agents are completed. This does allow the patient another recourse for remission. We hope to carry these studies further to include allogeneic and autologous grafts during periods of intensive chemotherapy.

4. Platelet Transfusions

The treatment of hemorrhagic complications of leukemia due to thrombocytopenia represent an important part of the Leukemia Service's activities. The studies in the Leukemia Service demonstrate the importance of the HLA genotyping of patients requiring long term support. In addition the relationship of granulocyte compatability of HLA antigens is the same as that observed with platelets. The technique of direct lymphocyte cytotoxicity has been used to assay for evidence of recipient sensitization. Selecting compatible random donors of HLA type have been successful by finding individuals who have similar HLA antigens and nonreactivity. However, transfusions of these platelets has not resulted in normal recoveries. Further studies will include the prevention of immunization by using single donor support and also the extraction of sensitizing leukocytes from transfused blood by leukopack and buffy coat removal. It may be possible to decrease the numbers of platelet transfusions by using more HLA compatible donors and single donor transfusions.

5. Search for Leukemic Antigens

The existence of specific tumor antigens in leukemic cells has been inferred for many years. During the past year, detailed attempts to examine the cells of leukemic patients with several in vitro tests, including mixed leukocyte culture, cellular cytotoxicity, and delayed hypersensitivity reactions to membranes have been initiated. This involves the obtaining of cells from patients on admission to the Leukemia Service and storage in liquid nitrogen. To date, cells have been harvested in 70 patients. At various time intervals cells are thawed and peripheral blood lymphocytes are drawn from the patient tested for reactivity against the autologous blast. Membrane preparations of relapsed or remission cells are used to test for intradermal reactivity. To date, over 100 assays have been performed. Cellular cytotoxicity assay has been positive in about a third of the patients, the mixed

Leukocyte culture in about one-half of the patients, in skin tests, in about 70% of the patients. Skin tests did seem to be the most correlated with remission, being positive in 13 of 16 patients as compared to two to five patients in relapse. These data are quite similar to the data obtained with Burkitt's lymphoma.

6. Determination of Therapeutic Activity in Acute Leukemia

The original intensive four drug combination, POMP, two of the original 35 children receiving POMP induction for acute lymphoblastic leukemia remain in continuous remission for 5-1/2 years.

Protocol 70-2 compares POMP plus asparaginase versus combination of prednisolone, cytosine arabinoside, daunorubicin, vincristine and asparaginase in AML patients. For adults the overall complete remission rate is 38%, the median duration of complete remission was 83+ days. For children similarly treated, 10 of 11 or 91% entered complete remission, lasting a median of 260+ days. The results in children have been markedly superior to those previously reported by our group and others.

In the past year, studies of several new therapeutic modalities have been instituted. The studies indicate that azotomycin in combination with asparaginase results in intolerable toxicity and inability of the patient to take the drug. To date no response has been seen with dibromodolcitol, PTG, bleomycin or Poly I-C in about 30 patients.

Analysis of the activities of the Leukemia Service in ALL with the combination chemotherapy protocol POMP since 1963 have indicated that the maximum advantage of the four drug combination has been achieved. Median duration of remission for these patients was about 13.5 months; 92% of these patients can be reinduced with subsequent chemotherapy and the survival median is 33 months with 20% of the patients alive and well for more than five years. The addition of BCNU following six months of intensive chemotherapy did not increase the remission duration. Therefore, it is the feeling of the Service that the maximum effect of POMP has been reached, and despite maximum chemotherapy, leukemia cells persist in the majority of patients. It is also recognized that prophylactic treatment of the central nervous system has not been employed in these regimens and that future protocols should include this as well as continued maintenance therapy.

7. Hodgkin's Disease

Five year follow-up is now available in the original combination chemotherapy of Hodgkin's disease. Sixty percent of the entire group is alive after five years. Of the complete responders 77% are alive after five years and 41% are continuously free of disease without therapy after the first six months of treatment. Since 1967 complete responders have been allocated to three different regimens: (1) no further therapy, (2) intermittent treatment with drug combination for an additional six

to 15 months, and (3) intermittent treatment with the new agent BCNU. Results should be made available next year to study the effects of continued therapy on this highly successful chemotherapeutic program.

Other findings of interest in Hodgkin's disease have included the demonstration that (1) Patients previously treated with radiotherapy who recur can be successfully treated with the multiple drug combination; (2) That the patients with Stage III disease treated with combination chemotherapy have a better survival and response than those treated with total nodal radiotherapy; (3) That BCNU has actively activity in patients who are resistant to alkylating agents. We have also shown that Hodgkin's disease is responsive to bleomycin given in a five-day course.

8. Lymphosarcoma

Since 1967 the randomized control clinical trial of optimal chemotherapy versus optimal radiotherapy in patients with Stage III and IV disease have been underway. Drug approach has involved the use of a three drug combination of cytoxan, vincristine and prednisone. Thirty-five patients have been evaluated, 57 have achieved complete remission; the median survival and duration has not yet been reached. The program appears to be very effective.

9. Carcinoma of the Breast

The adjuvant chemotherapy protocol using phenylalanine mustard has been activated and is in existence now. It has been adopted by the Eastern Cooperative Oncology Group and the National Surgical Breast Program. Whereas we have not been able to get enough cases at the Institute, the study should now be rapidly done by these two large cooperating institutions. In addition, a multiple drug combination chemotherapy protocol has been started utilizing the principles learned from the previous Hodgkin's treatment. These have included the use of combination chemotherapy intermittently allowing for host and marrow recovery between treatments. The study to date has indicated that combination of 5-fluorouracil, methotrexate, prednisone and cyclophosphamide is tolerable when given in 14-day schedules with an intermittent and treatment period of two days. This study will also be expanded and included in the Eastern Cooperative Oncology Group studying several possible alternatives.

10. Burkitt's Tumor

One hundred and twenty patients with Burkitt's tumor were admitted to the Lymphoma Treatment Center in the past 3-1/2 years; the first 57 were treated initially with one or six doses of cyclophosphamide in randomized trial. This trial indicated that in patients with Stage I and II disease a single dose of cyclophosphamide was sufficient to induce durable and long-term complete remissions. Patients responded more significantly to intensive chemotherapy with six courses compared to one course, however, in patients with Stage IV disease were refractory to

cyclophosphamide. Subsequently a combination of cytoxan, vincristine, methotrexate and cytosine arabinoside was employed; results indicate to date that this is a highly effective way of inducing complete remissions, however, patients have relapsed on this regimen. Central nervous system continues to be a major therapeutic problem in Burkitt's lymphoma, several clinical trials have been undertaken including prophylactic intrathecal chemotherapy which has not been shown to be effective. In addition studies of methotrexate or cytoxine arabinoside given daily for 10 days have proven not to be effective. The present protocol involves the use of a combination of methotrexate and cytoxine intrathecally with hydroxyurea orally.

It is of interest that the survival of the 92 treated evaluable patients reveals that clinical staging is still the best prognostic indicator. At the present time patients with Stage I and II disease have a two year survival or 82%. Those patients with Stage III disease have a survival of 64% and for central nervous system, bone marrow involvement or Stage IV have 0.

11. Lung Cancer

A major emphasis has been placed on the intensive treatment of patients with bronchogenic carcinoma. A feasibility study which included 46 evaluable cases was completed in August, 1970. Twenty-seven patients had limited disease; 17 had extensive disease. The median survival of patients with limited disease is 300+ days and that of the extensive disease is 123 days. Responders lived considerably longer than non-responders. The total of 22 of 36 patients with measurable disease had tumor shrinkage. Response rates were highest in patients with small cell carcinoma and adenocarcinoma. Since then a new study was started, consisting of a combination of nitrogen mustard and methotrexate with radiotherapy in patients with limited disease. At present 41 patients have been admitted to study. The activities of the lung cancer program should expand significantly by the addition of other hospitals and also through the activities of the Eastern Cooperative Oncology Group.

12. Hepatocellular Carcinoma

Systemic chemotherapy either with DCM or a combination of BCNU and 5-FU has been administered to 17 patients with hepatocellular carcinoma. In the 13 patients receiving dichloromethotrexate no objective tumor response has been seen. Seven patients have received the combination of BCNU and 5-FU, none of these patients have had objective tumor response and all died within three months of the start of treatment. Thus hepatocellular carcinoma remains a difficult disease to treat.

13. Malignant Melanoma

Thirty-seven patients with malignant melanoma were admitted during this year to the Solid Tumor Center in Africa. The patients were treated with NSC 45388 (dimethylimidazole carboxamide) only one dramatic tumor

response occurred in 12 patients. Of the seven patients treated with a combination of BCNU and vincristine, one patient has had a dramatic clearing of sensorium and regression of skin nodules after one course of therapy, but the response was short-lived and the patient died.

14. Cell Kinetics

Information derived from animal tumor systems has clearly shown that the knowledge of the tumor cell growth kinetics allows one to predict or improve therapeutic responses. The objective of this program is to examine methodology both experimentally and in humans of the cell cycle characteristics of the disease and following chemotherapy. In the past year several important observations have been made. These include the demonstration that the cell cycle characteristics of L1210 can be altered by administration of the nitrosoureas. This has resulted in a prolonged S phase and a lower growth fraction. In man the in vivo double labelling technique has allowed the development of rapid cell kinetic information. Fifteen patients have been studied with this technique in patients with solid tumors, and data have consistently shown that the S phases are fairly comparable in 19 to 25 hours. Also there is a significant variability in the mean cell cycle time resulting in dampening o a second PL curve.

The growth fraction of skin lesions is surprisingly high and may account for the skin lesions which respond to treatment more often.

In Burkitt's tumor cell kinetic information has been obtained by Professor Iverson and Dr. Ziegler, results indicate that there is a cell cycle time of approximately 22 hours an equal fraction approaching unity. This information is consistent with results of other in vitro kinetic information indicating that Burkitt's lymphoma is the fastest growing human neoplasm which may account for its extraordinary sensitivity to chemotherapeutic agents. Studies of cell kinetics of tumor in the central nervous system have also been completed.

In lung cancer, labelling indices have been done in marrows involved with tumor cells. The labelling indices were approximately 18%.

15. Studies of Minimal Residual Disease

Results and investigation in the tumor specific cell mediated immunity in 30 patients with Burkitt's have indicated that positive response to skin tests with tumor cell extracts are most likely to occur in patients who have had effective antitumor responses and is correlated with relatively long remission durations. There is a lack of a correlation between the cutaneous reactivity to autologous tumor extracts and the presence of central nervous system disease. Moreover, studies in Africa of the chromosome analysis of tumor tissue have indicated that the initial tissue is relatively euploid and normal in appearance. Recurrent tumors are also being studied to determine whether this presents recrudescence

of the original tumor cells and new disease derived from different cells. These studies are being expanded to patients with acute leukemia.

16. Alpha Fetoprotein

The results of serum alpha fetoprotein have indicated that this is a sensitive assay for disease. Highly quantitative methods are being developed by Dr. Robert McIntire of the Laboratory of Cell Biology, GL&C, and will be used to quantitate the serum levels. In addition proline hydroxylase has been shown to be significantly elevated in 71% of patients with hepatocellular carcinoma and may also serve to be a marker of minimal residual disease.

17. Assay of Marrow Stem Cells

The technique of marrow culture has been now well established in the Medicine Branch and can now be done with human marrow cells. This technique involves the culturing of marrow granulocyte precursors in vitro in a semisolid media containing methylcellulose and a stimulating factor. In man, approximately 50 colonies are formed following the plating of 200,000 cells. In patients with acute lymphocytic leukemia or acute myelocytic leukemia in relapse, zero to one colonies per 200,000 cells are formed. Following treatment with chemotherapy or induction of remission the colonies rise to normal levels. In the past year, we have been able to quantitate the proliferative state of the marrow cells by relating the number of colonies produced to the presence of granulocytes in the marrow. A linear relationship exists between the proportion of granulocytes plated and the number of colonies produced. In patients with aplastic anemia, acute lymphocytic leukemia and normals where differentials can be done, one can examine the proliferative capacity of marrow granulocytes. In normals approximately .05% of marrow granulocyte despite relatively small proportions of marrow granulocyte cells, 1.2% of the cells are producing colonies. This is experimental evidence indicating more marrow cells are in cycle. Moreover, by incorporating the cellularity of the marrow nucleated cell count per ml and the colonies formed per unit number of cells normals have about 2,000 to 10,000 colony producing cells per ml of marrow. Most patients with acute leukemia in relapse have relatively few. The technique therefore allows a quantitative assay of marrow granulocyte precursors, their proliferative capability and function. Studies are continuing to develop better methods for chemotherapy scheduling. In addition this technique can be used to assay marrow following freezing.

Administrative Report

In last year's Administrative Report we mentioned the initiation of the Medical Oncology Fellowship Program. With the opening of the 30 bed lung cancer unit at the Veterans Administration Hospital this activity has allowed us to encourage four individuals to participate in this program. These individuals will rotate through the Veterans Administration Hospital and the

Medicine Branch of the National Cancer Institute. We have also recognized the need to develop a formal training program within the Institute itself. More individuals need to be trained to develop more senior individuals. Many of our Clinical Associates are requesting third year positions. With the recognition of the American Society of Clinical Oncology and the American Board of Internal Medicine of Oncology, we have a major resource for training medical oncologists. This will require more help for the Medicine Branch for the training. Clinical Associates should spend a third and fourth year with us and complete their training, certified, both in internal medicine and medical oncology. This will allow more of the young bright Clinical Associates to stay in medical oncology.

The second administrative point stresses the fact that with the Phase I compounds, the methodology has improved to the point where they can be done rapidly and quickly. Straight Phase II evaluations of drugs are not possible within our facilities. Therefore, we feel that the development of contracts such as the Mayo Clinic in colon cancer and breast cancer should be encouraged and closer cooperation with the groups to do the Phase II screening. I feel that a major effort in the next year will be the development of a master protocol. This should allow the detailing of observations, decisions, and results to be sure that the drugs are being adequately tested. The Eastern Cooperative Oncology Group has accepted disease orientation and Phase II protocols are being done in lung and breast cancer patients.

It is also increasingly clear that a special effort should be developed in breast cancer at the NCI. Activities of both the Leukemia Service and the Solid Tumor Service are heavily committed to clinical research programs that are important. They include treatment of lymphomas, supportive care and more recently an active interest in ovarian cancer. I would hope that in the next year we can come up with adequate space, both laboratory and beds, to start an effective breast cancer program. It should be very closely tied in with the surgical service so that the proper kinds of patients can be admitted for treatment.

Finally there is a continued need to stress the lack of laboratory and animal room space for the Medicine Branch. The average number of modules per senior investigator is one. There is at present no animal facility available to the Medicine Branch. Moreover, most senior investigators could use two technicians. At the present time not every senior person in the Medicine Branch has a technician. Our laboratory programs are severely compromised. I would hope that within the next year that the space of the Medicine Branch can be adequately expanded.

Honors and Awards:

Paul P. Carbone

1. "Advances in Chemotherapy." Presented at the Pennsylvania State University, Hershey, Pennsylvania, October 197 .

2. "Combination Chemotherapy in Hodgkin's Disease." Presented at the Boerhaave Course in Leiden, The Netherlands, September 1970.

3. "Asparaginase: Clinical Trials." Chaired session at the International Symposium on Asparaginase, Paris, France, November 1970.

4. "Controlled Clinical Trials." Presented at the American Society of Hematology Meeting, San Juan, Peurto Rico, December 1970.

5. "Myeloma." Guest lecturer at the American Society of Hematology, San Juan, Peurto Rico, December 1970.

6. "Bone Marrow Culture." Presented at Hematology Seminar at Georgetown University Hospital, Washington, D.C., January 1971.

7. "Present Status of Cancer Chemotherapy." Presented at Biometry Seminar at the University of Buffalo, Buffalo, New York, January 1971.

8. "Leukemia and Allied Diseases." Presented at Introductory to Clinical Medicine at Georgetown University Hospital, Washington, D.C., February 1971.

9. "Non-Hodgkin's Lymphoma." Presented at the American College of Physicians Cancer Chemotherapy Course, Houston, Texas, March 1971.

10. "Advances in Hematology." Presented at the American College of Physicians Seminar, New York Hospital, New York, New York, April 1971.

11. "Burkitt's Tumor: Clinical and Immunologic Features." Memorial Hospital, New York, New York, April 1971.

12. "Burkitt's Tumor." Presented at Grand Rounds, Georgetown University Hospital, Washington, D.C., May 1971.

13. "Lymphoma." Presented at Sibley Hospital, Washington, D.C., June 1971.

14. "Burkitt's Tumor: Clinical and Immunologic Studies." Presented at Yale University, New Haven, Connecticut, May 1971.

15. "Combination Chemotherapy in Lymphomas - Phase I Studies." Presented at the EORTC Course in Clinical Pharmacology, Brussels, Belgium, May 1971.

16. Appointed Diagnosis and Therapy Committee, American Cancer Society.

17. Appointed to Staging and End Results Committee, American Joint Committee.

18. Appointed to Credentials Committee, Chairman, NIH.

19. Breast Cancer Task Force Member

20. Appointed Chairman, American Cancer Society-National Cancer Institute Chairman, Conference on Staging.

21. Appointed Clinical Professor of Medicine, Georgetown University Hospital.

22. Elected Chairman, Eastern Cooperative Oncology Group.

23. Elected Vice-President and President-Elect, American Society of Clinical Oncology.

Publications:

1. Brown, C.H.III, Ziegler, J.L., Templeton, C. and Carbone, P.P.: Cyclophosphamide toxicity in Ugandan and American patients with malignant lymphoma. Cancer 26: 462-467, 1970.

2. Ziegler, J.L., Morrow, R.H., Fass, L., Kyalwazi, S.K., Carbone, P.P.: Treatment of Burkitt's tumor with cyclophosphamide. Cancer 26: 474-484, 1970.

3. Brown, C.H., III, Canellos, G.P. and Carbone, P.P.: Effect of L-asparaginase on mouse bone marrow, assayed by in vitro culture. Blood 36: 385-389, 1970.

4. Cohen, M.H., Hirshaut, Y., Stevens, D., Hull, E.W., Davis, J.H., and Carbone, P.P.: Infectious mononucleosis followed by Burkitt's tumor. Ann. Intern. Med. 73: 591-593, 1970.

5. Carbone, P.P., Frost, J.K., Feinstein, A.R., Higgins, Jr., G.A., and Selawry, O.S.: Lung Cancer: Perspectives and Prospects. Ann. Intern. Med. 1003-1024, 1970.

6. Hull, E.W., Smith, C.F., Moertel, C.G., O'Gara, R.W., and Carbone, P.P.: Experimental studies of a-fetoprotein in man and monkey. In Burdette, W.J. (Ed.): Carcinoma of the Colon and Antecedent Epithelium. Springfield, Illinois, Charles C. Thomas, 1970, pp. 158-165.

Serial No. NCI-3423
1. Medicine Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Evaluation of Hemopoietic Stem Cell Function Using an In Vitro Bone Marrow Culture Method

Previous Serial Number: Same

Principal Investigators: Paul P. Carbone and John Northup

Other Investigators: George P. Canellos and Joan M. Bull

Cooperating Units: Solid Tumor Service, MB, NCI

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives:

1. Human granulocytic precursors can form colonies (IVCFU) in vitro using the method of Till and McCulloch. However, quantitative estimates of the IVCFU per cc of marrow, reflecting the total cellularity, the differential, and the proliferative capacity, have not previously been described. In a study, bone marrow, obtained from seven normal volunteers by aspiration of 4 cc was sedimented for two hours. The white blood counts were decanted, washed twice, and plated in enriched methylcellulose medium containing an extract from human embryo kidney cells as a stimulating factor. WBC counts and differentials were performed on the aspirate and the washed WBC button prior to plating. The amount of cells plated and the number of colonies per plate was linear. The mediun number of nucleated cells/ml was 4.8×10^7 (range: 2.9-7.2), immature granulocytes/ml, 2.2×10^7 (range: 1.1-3.2), and colonies per 200,000 cells plated, 47.0 (range: 21.3-116.5). The median number of colonies per 200,000 immature cells plated was 100.5 (range: 42/8-157.4), and number of colonies per ml of marrow was 12,953 (range: 4,680-20,046).

   Quantitation of the marrow granulocytic precursor pool can be done by using the in vitro colony forming assay, the marrow differentials, and the cell count per cc of marrow. In normals, corrections for the marrow differential and the differential of washed marrow cells caused up to 20% change in the value of IVCFU per cc of marrow. In abnormal and post-

treatment states, the effect of cellularity and of the proliferative capacity is more pronounced.

2. Mouse and human granulocyte precursors can be induced to form colonies in vitro using the method of Till and McCulloch. A specific "colony-stimulating-factor" is a necessary constituent of the medium. In this study, sera from mice and from humans with various hematologic disorders have been investigated for the presence of this factor.

   Mice were given cytoxan, IP, 200 mg/kg. Periodic serum samples were assayed for colony stimulating activity against pooled marrow from normal mice. Four days following treatment the mean baseline colony count rose from 14.50 to 49.50 colonies per 75,000 marrow white cells plated. This coincided with the nadir of marrow leukopenia.

   Sera from normal volunteers (2), and from patients with leukemia (2) or with aplastic anemia (1), when tested against marrow from normal volunteers, caused a rise from 0.58 to 11.41 mean number of colonies per 200,000 cells plated.

   These experiments provide further evidence for the presence of a humoral factor regulating granulopoiesis.

3. Use of marrow culture technique to assay frozen marrow and to follow patients with leukemia on chemotherapy.

   a. This technique is readily applicable to the quantitative assay of marrow granulocyte precursors of patients on chemotherapy. In the past year assays were done repeatedly in seven patients with acute leukemia following them through the initial marrow through periods of treatment and into remission. This technique allows the quantitation of marrow stem cell precursors based on colonies per ml and also an assay of the proliferative capacity of the marrow. It has been shown that patients initially have very few colonies per ml of marrow and as the patient is treated and the proportion of the marrow with leukemia decreases, the number of granulocyte precursors increase in the marrow. This is not necessarily associated with an increase in the marrow cellularity or proportion of granulocyte precursors but is related to the proliferative capacity of individual marrow and mature granulocytes. Ordinarily 0.05% of the marrow granulocyte precursors in normals can be induced to form colonies. In some patients with acute leukemia almost 1.2% of the marrow granulocytes were producing colonies. This is good evidence to indicate that a greater proportion of the marrow stem cells were in active cycle at this time.

   b. Using this technique we hope to develop quantitative estimates of marrow viability following various procedures of freezing. This technique can be used to assure that frozen marrow will have the capability of functioning in vivo.

c. Morphology. Techniques have now been developed to examine the cells from culture both histologically and histochemically. Histologically, in proportion the cells appear to be granulocytes or mononuclear cells some of which appear to be frankly phagocytic and monocytoid in feature. Recent techniques have also been developed using marrow grown in fibrinogen which can be subsequently clotted and prepared for electron microscopy. Very nice pictures of the granulocytes can be developed this way. Finally, histochemical stains have indicated that a proportion of these cells are producing myeloperoxidase.

Future Course of Project:

Next year the plan is to have two individuals working in the laboratory on this project, and we will be concentrated on assaying granulocyte precursor cells in patients receiving intensive chemotherapy hoping to arrive at better treatment schedules. In addition, a continued attempt will be made to try to differentially grow leukemic cells hoping to develop an assay for minimal residual leukemia. We feel that this may be possible because certain leukemic patients appear to produce colonies that are distinctly abnormal, more clumped, than the normal granulocyte colonies. It is hoped that we can arrive at methods of doing this routinely in patients with leukemia.

Honors and Awards:

Dr. Paul P. Carbone

1. "In Vitro Studies of Mouse and Human Marrow Growth." Presented at Georgetown University Hospital, January 1971.

Dr. John Northup

1. "Quantitation of Marrow Granulocyte Precursors." Presented at the Annual Meeting of the American Federation for Clinical Research, May 1971.

2. "Effect of Serum Factors on In Vitro Granulocytic Colony Formation." Presented at the Sixth Leucocyte Culture Conference, June 1971.

Publications:

1. Brown, C.H. III, and Carbone, P.P.: In vitro growth of bone marrow from normal human subjects and patients with leukemia using an enriched methylcellulose medium. J.N.C.I. (in press).

2. Brown, C.H. III, and Carbone, P.P.: Effects of chemotherapeutic agents on normal mouse bone marrow grown in vitro. Cancer Res. 185-190, 1971.

Serial No. NCI-3400
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Chemotherapy of Acute Leukemia

Previous Serial Number: Same

Principal Investigators: Edward S. Henderson, M.D., Brigid G. Leventhal, M.D. Ronald A. Yankee, M.D., Arthur S. Levine, M.D., and Robert Graw, M.D.

Other Investigators: Jacqueline Whang-Peng, M.D., Roger Halterman, M.D., Geoffrey Herzig, M.D., Harvey Gralnick, M.D., and John Bennett, M.D.

Cooperating Units: Acute Leukemia Cooperative Group B
Chemotherapy Service, Washington VA Hospital
Baltimore Cancer Research Center
Laboratory of Chemical Pharmacology, NCI
Human Tumor Cell Biology Branch, NCI
Hematology Service, Clinical Pharmacology, CC
Rochester University School of Medicine

Man Years:

Total: 6½*
Professional: 4½*
Other: 2

* Exclusive of first-year Clinical Associates

Project Description:

Objectives:

1) To assess effectiveness against acute leukemia of new candidate drugs.

2) To improve the effectiveness in leukemia of compounds with known activity by improved scheduling or combination administration.

3) To relate clinical effects with pre-clinical pharmacology, activity, toxicology, and model systems in order to test screening programs and models, and in order to develop more effective clinical programs for the arrest and cure of leukemia.

4) To study the natural history and treatment modified course and biology of leukemia in order to identify factors of predictive and prognostic significance, and define variables which must be taken into consideration in the design and evaluation of treatment protocols.

5) To interrelate cytotoxic drug approaches to leukemia with adjunctive measures, e.g., irradiation, immunotherapy, reverse transcriptase inhibitors, for greater control or cure of acute leukemia.

Major Findings

I. Effect of Multiple Drug Combinations

A) Original POMP: Two of the original 35 children receiving POMP induction for acute lymphoblastic leukemia (ALL) have enjoyed continuous remission for more than 5½ years without further therapy. Seven of 35 (20%) survived with or without relapses for five years or over.

Two of the original 40 patients with acute myelocytic leukemia (AML) treated by POMP have been in continuous remission for seven years.

B) "New POMP" LS 68-8: This study in childhood ALL was designed to test the effectiveness of repeated five day courses of prednisone, methotrexate, vincristine, and 6-mercaptopurine, given at the same dose as in the earlier POMP program, but at 2-week rather than 4-week intervals. This schedule is identical to that used during remission induction, and similar to that employed by the Acute Leukemia Cooperative Group B in Protocols 6601 and 6801, which, however, consisted of one or two drug basic maintenance, rather than simultaneous administration of four drugs. At the end of six months (12 courses) of maintenance POMP, all patients received BCNU 50 mg/m^2 for three days, following which the patients remaining in complete remission were randomly assigned either no further therapy or six additional courses of POMP during the subsequent six months.

The results of this protocol have been highly discouraging but of great importance, as noted in the following Table LS 68-8.

Patients entered: 67 (35 boys, 32 girls; median age 6 (< 1-19) years)

Patients achieving complete remissions: 61/67 (91%)

Patients in consolidation phase (POMP for 6 months + BCNU):
relapsed during interval: 12 (1 death)
uncomplete (still in CR): 10

Patients randomized post BCNU: 39

no further treatment: 19 patients
12 boys/7 girls
median total remission: 350 days
6 months additional POMP: 20 patients
6 boys, 14 girls
median total remission: 450 days
still in remission: 9/20

Remission duration for all patients (including relapses pre- and post-BCNU therapy) 301 days. Patients remitting on same drugs (POMP after relapse from LS 68-8: 15/29 = 53%.

This extension of POMP therapy was less successful than the initial studies of POMP in terms of remission duration and re-induction. Since the frequency of intensive therapy in remission was similar to that of protocols 6601 and 6801 of Acute Leukemia Group B, which also had more favorable remission durations, the discrepancy appears to result from the frequency of vincristine and/or prednisone administration. It appears clear from our experience with VAMP, POMP, and now LS 68-8 each sequentially more intensive in dose or schedule that we have passed the point of maximum responsiveness with this four drug combination. Following randomization, six additional months of therapy appears to provide a duration of remission superior to that associated with no further therapy, although these differences are not yet statistically significant.

II. Comparative Trials of Combination Chemotherapy for Adult ALL (in collaboration with Acute Leukemia Cooperative Group B):

Protocol 6812 and 6812A have sought to compare the effectiveness of vincristine, prednisone, and daunorubicin (VDP) versus POMP in remission induction and subsequent unmaintained remission duration. Because of the short duration of complete remission following either combination, a maintenance program was added, consisting of monthly 5-day courses of methotrexate plus periodic inducer doses of prednisone and vincristine (following VDP induction) or monthly POMP (following POMP induction). The results of this study are as follows:

	No. of Patients Evaluated	% Complete Remission	Median Duration Complete Remissions (Weeks)	Median Survival (Months)
VDP	31	48	11	10
POMP	24	58	12	6

The effect of remission maintenance was as follows:
Total patients maintained: 120 days median remission (25-570+)
Total patients unmaintained: 49 days median remission (17-1802+)

Analysis of this data indicates the superiority of remission maintenance in adult ALL and the lack of significant differences in response between patients treated with either induction regimens.

Because of the relatively low remission induction rate for 6812 and in light of the increased duration of remissions observed in Acute Leukemia Group B study 6801 of children with ALL receiving asparaginase during remission induction, asparaginase was recently added to VDP and POMP in the adult ALL protocol. Very preliminary results indicate increased toxicity for the regimen without comparable increases in the remission induction rate (8/18 complete remissions to date.)

III. Combination Chemotherapy of Adult and Childhood Acute Myelocytic Leukemia:

Since November 1970, 24 adults and 11 children have been entered into LS 70-2 (POMP-PRAVDA) as initial remission induction therapy. This protocol compares POMP plus asparaginase; PRAVDA (prednisone, cytosine arabinoside; daunorubicin, vincristine, and asparaginase); and alternating courses of the two regimens for remission induction and maintenance. For adults the overall complete remission rate was 9/24 or 38 percent with no significant differences among the three regimens. Of the 15 patients who did not remit, 9 died of hemorrhage and/or infection. The median duration of complete remission is to date 83+ days (range 12-416).

Of the children similarly treated 10/11 (91%) have achieved complete remissions lasting a median of 160+ days (range 26+ to 317+). The single non-responding child died during attempted induction. These results must be compared to our previous remission rates with POMP (without asparaginase) of 35-50% for adult AML and 75% in childhood AML. Asparaginase toxicity occurred frequently in both children and adults and included pancreatitis usually associated with thrombophlebitis (18% of cases) hepatitis (18%) and anaphylactic shock (31% of cases). Only one-third of the patients entered have been able to tolerate more than three doses of intermittent asparaginase, despite concurrent administration of immunosuppressive antimetabolites and high dose prednisone.

IV. Prognostic Implications of Laboratory Studies in Acute Myelocytic Leukemia:

A) An analysis of cytogenetic abnormalities in 103 patients with acute myelocytic leukemia has indicated that the median survival of the 72 percent with anueploid or pseudodiploid malignant cell lines and the 30 percent without cytogenetic abnormalities were similar at 9.0-9.5 months. However, 7 patients without abnormalities survived 26 months or longer, whereas all patients with abnormal karyotypes had died by the 25th month.

B) In a study of AML patients with or without Auer rods identifiable in their malignant myeloblasts, the response rate and survival of the two groups were significantly disparate. Patients with Auer

rods survived an average (median) of 7.5 months, while Auer rod negative patients survived only 4.0 months from diagnosis.

C) Asparaginase plus glutamine antagonists in acute lymphocytic leukemia (LS 70-1).

This study was based on animal data suggesting a synergistic interaction of asparaginase and compounds which alter glutamine metabolism. Earlier studies (see Annual Report 1969) of asparaginase plus the orally administered glutamine antagonist, azaserine, failed to augment response rates. Azaserine is relatively unstable in acid media, so that for the current study both asparaginase and azaserine have been given by the intravenous route.

All patients received asparaginase, 15,000 I.U./m^2 for three days out of every four for a total period of four weeks. The control group received no other medication, while the randomly determined test group received azaserine 50-150 mgm/m^2 q 4 d I.V., azaserine 300 mgm/m^2 q 4 d I.V., or azotomycin 5-15 mg/m^2 p.o. respectively.

Twenty-four patients were entered into this protocol with the following results:

	Complete remission	Remission duration (days)
Asparaginase alone:	3 of 5 patients	33-172
Asparaginase+low dose azaserine	1 of 5	200+
Asparaginase+high dose azaserine	6 of 9	96-172
Asparaginase+azotomycin	0 of 5	

For the groups as a whole, asparaginase was well-tolerated in 16 of 21 patients (76%). Asparaginase had to be discontinued in two cases because of hepatotoxicity and in three others because of severe hypersensitivity reactions. Azotomycin caused severe protracted nausea and vomiting in all five recipients and had to be discontinued in three instances. The overall complete remission rate (10/24) is consistent with that seen in comparable patients receiving asparaginase alone.

V. Effectiveness of new agents: Phase two evaluations are in progress with the following agents, listed together with response rates seen to date.

A) dibromodulcitol

maximum dose: 250 mgm/m^2/day X 3 days, or 1,000 mgm/m^2 q week

ALL: no remissions. Improvement in 1 of 6 (decreased bone marrow and peripheral blood blasts)

AML: no response in 2 patients

Toxicity: myelosuppression, slight nausea, and vomiting.

B) 4'Demethyl epipodophyllotoxin
Maximum dose: 100 mgm/m^2 q 4 d
ALL: 8
AML: 3
} reduction of peripheral blood and organ blast cells. No effect on bone marrow.
Toxicity: Pancytopenia

C) Poly I - Poly C
Maximum dose: 50 mgm/m^2 q d or q o d
ALL: no response in 6 patients
AML: no response in 2 patients
Toxicity: fever, anorexia

All higher dose level (25-50 mg/m^2) patients have demonstrated low-level induction of inteferon production.

Significance:

Combination chemotherapy has provided the best reported response rates, remission maintenance and survivals in adults and children with acute lymphocytic and myelocytic leukemia. The optimum doses, schedules, and interrelationship of the several drugs used in these combinations has not as yet been clarified. Studies of the Leukemia Service, NCI, of combinations of the four active agents vincristine, prednisone, 6-mercaptopurine, and methotrexate have been conducted since 1963. Successive protocols in childhood ALL have been designed to deliver larger quantities of drug per unit time and for larger periods of time, the evaluable parameters being complete remission (CR) induction rates, remission duration, rates of reinduction for relapsed patients, median survival, and percent of long term (5 year) survival. A comparison of these protocols is as follows:

Protocol	Years of acquisition	Duration of therapy	% CR	Frequency of Maintenance courses	Median duration of CR (months)	% Reinduction	Median Survival (Months)	≥ 5 year Survival
VAMP	1962-3	5 mos.	88	q 20-30 days	9.5	100	24	20
POMP	1963-5	14 mos.	92	q month	13.5	92	33	20
68-8	1968-71	12 mos.	91	q 2 weeks	12-14	50	?	?

The longer period of maintenance appears slightly superior to the short duration of the earliest protocol, but increasing the frequency of POMP courses

has not altered the rate of relapse and has induced apparent resistance to the remission inductive effects of vincristine, prednisone, 6-mercaptopurine, and methotrexate. Furthermore, the use of bis-B-chloraethyl-nitrosourea given at the maximally tolerable dose following six months of intensive chemotherapy (and at which leukemia cell residuals should theoretically have been sharply reduced), did not increase remission durations using historical controls as comparisons. These results indicate that a maximum effect of POMP has been reached, that further intensification of this particular regimen is not warranted, and that at maximal permissable dose levels leukemic cells persist in a majority of patients.

It should be noted that prophylactic treatment to the central nervous system was not employed in these regimens, and that the period of therapy was arbitrarily finite. Recent results from other institutions suggest that both CNS prophylaxis, and continuous maintenance after periods of early intensive therapy would result in prolongation of hematological remissions. Future studies should incorporate these approaches.

Combinations of asparaginase administered intermittently with combination steroid plus antimetabolite regimens have increased the toxicity of such regimens in both ALL and AML without substantially increasing the rate of response. Asparaginase alone is undeniably effective against ALL, does not appear additive or synergistic with glutamine antagonists, and appears of minimal utility against acute myelocytic leukemia.

POMP and PRAVDA have achieved remission frequencies comparable to the best protocols reported for adult AML and have initially provided better results in childhood AML (91%) than any previous study. Expansions of this regimen in childhood AML is necessary to confirm this preliminary observations and to associate the impact of this regimen on survival and curability. It is noteworthy that the response rate of PRAVDA derived from the individual activities of its component drugs and assuming independent activity of each agent is identical to the observed results in children (92 vs 91 percent), but not in adults (69 predicted vs 39% observed). This provides indirect evidence of the limiting effects of diminished host defenses and normal stem cell reserves in adults, which limit the effectiveness of active agents in the older age categories. Such limitations underline the need for improved support facilities in order to increase the yield of favorable responses to drug therapy in adult acute leukemia.

Proposed Course of Project:

The major chemotherapy efforts of the Leukemia Service will be to interdigitate the promising developments in chemotherapy, radiotherapy, immunotherapy, and optimal supportive majors toward a curative regimen for acute leukemia. The relative biological effects of each component shall be quantified where possible, and the most appropriate sequences, doses, and combinations assessed. Promising new compounds of unique activity (e.g., interferon inducers, membrane active agents, reverse transcriptase inhibitors) will be evaluated singly and

incorporated into the framework of established therapies. Prophylactic regimens against CNS residua, utilizing the best drug and irradiation techniques will be incorporated into the first line protocols for both adult and childhood acute leukemia. It is hoped that the program scope and staff will be expanded to provide cell kinetic surveillance for all therapy studies and the development and implementation of biochemical monitoring of drug susceptibilities to chemotherapeutic agents both at the initiation of therapy and serially throughout drug trials. The goal of this aspect of the leukemia therapy program, and the allied immunological and patient support programs, will clearly be toward leukemia cell eradication and clinical cure of this disease.

Honors and Awards: None

Invited Lectures:

Dr. Edward S. Henderson

1) Georgetown University School of Medicine, April, 1971.

2) George Washington University School of Medicine, February, 1971.

3) National Naval Medical Center Symposium in Cancer Therapy, 1970.

4) American College of Physicians Meeting; Chairman of Symposium: To treat or not to treat acute leukemia, 1970.

5) Guest professor; The Royal Albert Hospital, Melbourne, Australia, May, 1971.

6) Guest lecturer; The Pediatric Blood Club, Atlantic City, N.J., April, 1971.

7) Guest lecturer; The Asian-Pacific Congress, International Society of Hematology, Melbourne, Australia, May, 1971.

8) Instructor, Educational Sessions; American Society of Hematology, San Juan, Puerto Rico, December, 1970.

9) Visiting professor; Ontario Cancer Research Institute, Toronto, Canada, 1970

10) Visiting professor; Royal Marsden Hospital, Sutton, Surrey, England, July, 1970.

Publications:

1) Goodell, B., Leventhal, B.G., and Henderson, E.S.: Cytosine arabinoside treatment of acute myelocytic leukemia. Clinical Pharmacology and Therapeutics (in press).

2) Carbone, P.P., and Henderson, E.S.: New agents in the treatment of cancer. Recent Results in Cancer Research 30: 56, 1970.

3) Whang-Peng, J., Henderson, E.S., Knutsen, T., Freireich, E.J., and Gart, J. J.: Cytogenetic studies in acute myelocytic leukemia with special emphasis on the occurrence of the Ph^1 chromosome. Blood 36: 448, 1970.

4) Goldin, A., Sandberg, J.S., Henderson, E.S., Newman, J.W., Frei, E., III, Holland, J.K.: The chemotherapy of human and animal acute leukemia. Cancer Chemotherapy Reports (in press).

5) Leventhal, B.G., and Henderson, E.S.: Combination therapy of acute leukemia with asparaginase plus other cytostatics. Proc. Symposium International Sur La l-Asparaginase, 1970 (in press).

6) Henderson, E.S.: Acute lymphoblastic leukemia. In Cancer Medicine, Frei, E., III, and Holland, J.F., editors, Williams & Wilkins, 1971 (in press).

7) Carbone, P.P., Haskell, C.M., Canellos, G.B., Leventhal, B.G., Block, J., Serpick, A.A., and Selawry, O.S.: Early clinical and toxicology studies. Recent Results in Cancer Research 30: 46, 1970.

Serial No. NCI-3407
1. Medicine Branch
2. Leukemia Service
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: 1) Studies on the Genetic Organization of the Small Oncogenic Papovavirus, SV40

2) Studies on *in vivo* Tumorigenesis and *in vitro* Transformation Induced by DNA Tumor Viruses: Viral and Host Components as Assayed by Biochemical and Biophysical Techniques

3) Studies on the Molecular Mechanisms by Which Human Herpes Viruses Establish a Latent State: The Passenger Situation, Recurrent Infection, and Putative Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Arthur S. Levine, M.D.

Other Investigators: Stuart Siegel, M.D., Lowell Schnipper, M.D., Andrew Lewis, Jr., M.D., Myron Levin, M.D., Michael Oxman, M.D., Patrick Henry, M.D., Richard Samaha, M.D., Thomas Kelly, Jr., M.D.,Ph.D., Clyde Crumpacker, M.D., Cephus Patch, Ph.D., Normal Salzman, Ph.D., Dorothy Moore, M.S., Wallace Rowe, M.D., and John F. Enders, Ph.D.

Cooperating Units: Laboratory of Viral Diseases, NIAID: Lewis, Rowe, Crumpacker, Kelly

Laboratory of Biology of Viruses, NIAID: Patch, Salzman

Research Division of Infectious Diseases, Boston Children's Hospital Medical Center and Harvard Medical School: Enders, Oxman, Levin (Formerly Medicine Branch, NCI)

Department of Medicine, University of Missouri: Henry, Samaha (Both formerly Medicine Branch, NCI)

Man Years:

Total: 10
Professional: 9.5
Other: 0.5

Project Description:

Objectives:

1) To detect the presence of extremely small amounts of virus-specific nucleic acids in malignant cells, employing highly sensitive methods developed in this laboratory and elsewhere. Such assays may be positive in the absence of virus recovery and virus antigens.

2) To study the genetic organization of SV40 by an analysis of the varying segments of the SV40 genome contained within adenovirus-SV40 hybrid virus DNA molecules. Such studies employ biologic, biochemical, and biophysical techniques to arrive at a genetic map of an animal tumor virus.

3) To identify and isolate that gene(s) of SV40 which relate most directly to the virus' oncogenicity, employing the previously noted adenovirus-SV40 hybrid viruses.

4) To analyze the behavior of virus-transformed cells as a function of the molecular conformation of viral DNA within these cells, and as a function of events which may control viral genetic expression.

5) To analyze the behavior of permissive cells acutely infected by DNA viruses as a function of the molecular conformation of the viral nucleic acids and as a function of events which may control viral genetic expression.

6) To study aspects of animal cell transformation occurring spontaneously in vitro, without known exposure to oncogenic viruses.

7) To study the molecular mechanisms by which the human herpes viruses (H. Simplex, varicella-zoster, cytomegalovirus, and the Epstein-Barr virus) establish a latent state, as it relates to recurrent infection and to putative tumorigenicity. Such mechanisms will be approached with the techniques previously applied successfully to the smaller DNA tumor viruses.

Methods Employed:

Standard biochemical and radioisotopic methods have been employed for the measurement of nucleic acid and protein synthesis. Procedures for the extraction of viral and cell nucleic acids have been improved. New methods have been evolved which permit the in vitro synthesis of viral complimentary RNA of very high specific activity: this RNA has been used to detect extremely small amounts of viral DNA in malignant cells. Techniques for hybridization of viral and cellular nucleic acids have been improved, and modifications which permit more quantitative comparison of heterologous nucleotide sequences have been described. Viral DNA has been analyzed by equilibrium density centrifugation (neutral and alkaline), analytical ultracentrifugation, ethidium bromide banding of superhelical DNA, temperature-absorbance profiles, and electron microscopic techniques. Standard tissue culture techniques have been carried out, as well

as immunoflourescent assays for viral antigens in cultured cells. Localization of SV40 genes in adenovirus-SV40 hybrid DNA molecules has been performed by enzymatic digestion with selective nucleases, prepared in our laboratory with column chromotography, and by electron microscopic examination of heteroduplexes formed with one strand of wild-type adenovirus DNA and one strand of hybrid virus DNA.

Procedures for fractionating the chromosomes of virus-transformed cells according to group-size have been developed, as have hybridization methods to determine which chromosomes contain integrated viral genes.

Major Findings:

1) A series of genetically homologous SV40-exposed and unexposed hamster tumor lines was assayed for virus-specific DNA by methods which permit the detection of 1-2 viral genes in each tumor cell. It was found that "classically" transformed cells with surface (S) and neclear (T) viral-specific antigens contain SV40 DNA, but that transformed cells with only the viral S antigen contain no viral DNA. Since the S antigen appears to be a normal fetal hamster antigen, and since it appears in non-fetal hamster cells only after exposure to SV40, the results indicate that this virus may be able to induce a heritable dedifferentiated surface change by a hit-and-run mechanism. If confirmed, the results mean that virus can induce surface changes (and possibly oncogenicity) without persistent viral genetic information.

2) The first of a series of non-defective adenovirus 2-SV40 hybrid viruses was characterized in terms of the biological function and genetic conformation of the SV40 moeity. These viruses are exceedingly useful for biochemical and genetic study since they replicate without any helper particles and hence can be obtained as purified, homogeneous populations. Ad2 + ND1 virus contains the entire, or virtually the entire, adenovirus 2 genome covalently linked to 250,000 daltons of SV40 DNA; the recombinant DNA molecule is contained within an adenovirus 2 capsid. This amount of SV40 DNA is equivalent to one average or 2 small genes. The product of the SV40 moiety in ND1 virus has been identified: a perinuclear antigen ("U") which is heat-stable and synthesized early in the SV40 replicative cycle prior to viral DNA replication. The presence of this SV40 function may account for the fact that the hybrid virus grows normally in monkey cells, while wild-type adenovirus 2 does not. The Ad2 + ND1 virus has also induced transformation in vitro and hamster tumors in vivo, while wild-type adenovirus 2 does not. This oncogenic potential may reside within all or part of the SV40 gene (and be synonomous both with the perinuclear U antigen and with the hybrid virus' capacity to grow in monkey cells); alternatively, the adenovirus moeity of the hybrid may have undergone a subtle mutation which now renders it oncogenic. Studies to distinguish between these two possibilities are underway.

3) The SV40 moiety in Ad2 + ND1 virus has been compared with wild-type SV40 virus in terms of sequence homology of the SV40 specific RNAs induced in cells infected by the respective viruses. Techniques were developed for obtaining good yields of pure SV40 early RNA. By competitive hybridization, it was found that the early genes, i.e., those which are transcribed prior to viral DNA replication, comprise 35% of the entire wild-type SV40 genome, or 2-3 genes, out of 6-10. It was then found that the one SV40 messenger RNA in the ND1 virus is an early SV40 messenger, which accords with the early appearance of the perinuclear antigen and possibly with the evidence that oncogenicity is probably an early viral function.

4) Initial characterization was performed on 4 more nondefective adenovirus 2-SV40 hybrid viruses, each containing varying segments of the SV40 genome. As compared to the one SV40 messenger RNA induced by ND1 virus, ND2 induces approximately 2 messenger RNA species; ND3-A induces one-half; ND3-B induces one and one-half; ND4 induces 3-4 messenger RNA species. When compared with the early and late messenger RNA species of wild-type SV40 virus, ND2 induces approximately similar RNA species; ND3-A and ND3-B less than early, and ND4 more than early (probably one additional late function). The proteins induced by the SV40 moeities in these 4 nondefective viruses are: TSTA, as well as the U antigen, by ND2; none by ND3-A nor ND3-B; and TSTA, U, and T antigen by ND4. All of these viruses have induced transformation and tumors, while wild-type adenovirus 2 does not. As discussed in #2, this observation may mean that all 5 nondefective viruses so far isolated share an SV40 genetic sequence responsible for oncogenicity, and that this sequence is represented by the "one-half" messenger RNA within ND3-A; alternatively, as discussed in #2, it may mean that the adenovirus moeity in all of these nondefective hybrids has undergone a subtle mutation from the wild-type which now renders it oncogenic. A further observation of interest is the fact that no protein products of the very limited SV40 genome in ND3-A and the larger segment in ND3-B have been detected. One explanation is that there is not enough genetic information in ND3-A's SV40 moeity to code for an intact peptide, and that in ND3-B, an initiator piece is missing from the message such that translation does not occur.

5) Enzymatic digestion of the ND1 DNA molecule has been accomplished with identification of the site of SV40 linkage in the recombinant molecule: this site is 20-35% from the 3' end of the linear duplex, and the finding has been confirmed by direct electron microscopic examination of a renatured heteroduplex formed between one hybrid strand and one wild-type adenovirus 2 strand (Szybalski technique). The results of all of the studies of the non-defective hybrid viruses described in #2-5 demonstrate that genetic analysis ("mapping") of an animal tumor virus is presently feasible, and that the segments directly associated with oncogenicity can be identified and isolated.

6) Studies have also been accomplished on a defective adenovirus 2-SV40 hybrid virus (Ad2 + SV40, Var. HEY). It was shown that this virus, which efficiently yields wild-type SV40 virions during infection, and thus contains

the intact SV40 genome, has the following genetic conformation: covalent linkage of the SV40 and adenovirus DNA moeities, as with the non-defective hybrids. Further, it was found that in the plaque-purified AD2 + SV40 (HEY) virus preparation, there are no adenovirus capsids containing free, circular SV40 DNA (wild-type). A second defective adenovirus 2-SV40 hybrid which also has the intact SV40 genome but yields SV40 virions with low efficiency (LEY) is under study.

7) Procedures have been developed which permit the fractionation of chromosomes by size-group in virus-transformed hamster cells. Our evidence is that fractions can be obtained with morphologic purity and that DNA can be extracted from these chromosomes in sufficient yield and quality to permit hybridization with complimentary viral RNA. We have found situations in which viral DNA is contained within only one size-group of the chromosomes, and other situations in which it is located diffusely.

8) SV40-transformed hamster heart cells which differ significantly in their ability to induce tumors were examined. It was found that there are 3 copies of the SV40 genome in a high-oncogenicity line and 2 copies in a low-oncogenicity line, a difference not considered significant. Preliminary experiments suggest rather that differences in the species of viral messenger RNA transcribed in the 2 lines may be associated with their differing biological behavior.

9) Hybridizable RNA was studied by competitive hybridization using primary hamster embryo cells (not serially propogated) and continuous lines of virus-transformed, spontaneously oncogenic, and contact-inhibited (non-oncogenic) cells. It was found that both transformed and untransformed continuous lines contain a significant number of additional hybridizable hamster RNA sequences not found in primary cells, but that the additional sequences cannot be differentiated in transformed and untransformed continuous lines. These results indicate that 1) additional RNA species do not correlate necessarily with the acquisition of oncogenicity, although their appearance may be an early prelude; 2) altered transcription in contact-inhibited continuous lines may be due to the absence of extrinsic restraints present in vivo, or to the emergence of one or a few cell types from an unselected mixed primary cell preparation. (The additional RNA species previously reported in primary neoplastic cells as compared with their normal controls may be due to insensitivity to extrinsic restraints in vivo, rather than to absence of these restraints as in tissue culture.) The significant number of new RNA species in contact-inhibited cells suggests that these cells may also contain many new proteins. While their structure and function appear grossly normal, cultured cells may contain many enzyme activities, etc., present in tumor cells, but absent in truly normal primary cells.

Proposed Course

1) The four non-defective adenovirus.2-SV40 hybrid viruses initially charact-

erized will be analyzed in greater detail. The molecular weight, base composition, linkage, and temperature-absorbance profiles of the recombinant genomes will be determined, as will the percentage and site relationship of the SV40 and adenovirus DNA moieties. An attempt will be made to learn how the function of these varying SV40 genetic segments is controlled by the contiguous adenovirus genes. We also plan to isolate the SV40 genes in the hybrid molecules and to introduce them in cell-free protein synthesizing systems so that their individual products can be identified and compared with new proteins found in SV40-transformed cells. Complementation tests employing various mixtures of the hybrid viruses are underway. Attempts will be made to isolate and purify other non-defective adenovirus 2-SV40 hybrid viruses which may contain segments of the SV40 genome not present in the 5 viruses so far studied. The long-range goal of this work is to completely map an animal tumor virus genetically, to identify and isolate the gene (s) associated with oncogenicity, and to learn what the product of this gene is, and what is the molecular mechanism of its activity.

2) Our study of high and low oncogenicity lines of SV40-transformed hamster cells will be completed, and the approach will be extended to other virus-transformed lines which show a similar variance in biological behavior. An attempt will be made to correlate differences and similarities in viral gene content, extent of transcription, and viral protein translation.

3) Our study of defective adenovirus 2-SV40 hybrid viruses which differ in their ability to yield wild-type intact SV40 virions during acute infection will also be completed. The genomes of the HEY and LEY viruses will be examined by electron microscopy, using the Szybalski technique of renatured heteroduplex formation. The results should indicate whether the HEY virus contains more than one copy of the SV40 genome, thus explaining a greater frequency of excision and recombination. The difference in efficiency of SV40 yielding could also be explained by varying adenovirus control over SV40 DNA transcription or translation, and these possibilities will be explored.

4) Experiments involving fractionation of chromosomes from tumor cells will continue, with identification of those chromosomes in which viral genes are integrated. When the system yields consistent results with in vitro hamster cells, it will be applied to human situations such as the trisomies, Fanconi's syndrome, and chronic myelocytic leukemia. These are situations in which malignancy is present or is highly likely to appear, and in which a high frequency of in vitro virus transformation can be demonstrated. It will be of interest to learn if a correlation can be made between chromosomal anomaly and location of viral genes.

5) We shall undertake preliminary experiments to learn if the herpes viruses can be purified sufficiently to permit resolution of viral DNA and any host DNA which may be encapsidated during infection. If pure herpes DNA can in fact be obtained, or can be refined by techniques which may seper-

ate host from viral DNA, a wide variety of experiments are possible. It is planned to have these viruses purified and supplied by Dr. Clyde Goodheart, Chicago, who has had extensive experience with the herpes viruses.

Following are some of the many situations which might be approached using purified herpes DNA, or complimentary RNA generated in vitro from a pure herpes DNA template, as probes to detect the homologous viral nucleic acids in cells which otherwise give no indication of the viral presence. H. Simplex, type 2, in human cervical carcinoma cells; EBV in non-producing Burkitt cells (already demonstrated by Harld zur Hausen, but never confirmed); H. samurai in New World monkey lymphoma and acute lymphocytic leukemia; H. Simplex, type 1, in recurrent skin lesions (during latency); varicella-zoster during latency; and cytomegalovirus during latency (e.g. in blood bank preparations). The initial approach in all these situations would be to learn if in fact the viral nucleic acids can be detected in the absence of any other morphological or biological evidence of the viral presence. If so, what are the molecular events which explain why a herpes virus remains latent, as contrasted with the events occuring during frank infection; finally, what is the molecular basis for acute infection in one cell type by a herpes virus, as contrasted with tumorigenicity in a different cell type by the same virus.

Honors and Awards

1) "Studies on the genetic organization of the small oncogenic virus, Simian Virus 40," lecture delivered at the University of Vermont, April 2, 1971, Arthur S. Levine, M.D.

Publications

1) Levine, A.S., Oxman, M.N., Henry, P.H., Levin, M.J., Diamandopoulos, G.T., and Enders, J.F.: Virus-specific deoxyribonucleic acid in simian virus 40-exposed hamster cells: Correlation with S and T antigens. J. Virology 6: 199-207, 1970.

2) Crumpacker, C.S., Levin, M.J., Wiese, W.H., Lewis, A.M., Jr., and Rowe, W.P.: Adenovirus type 2-simian virus 40 hybrid population: Evidence for a hybrid deoxyribonucleic acid molecule and the absence of adenovirus encapsidated circular simian virus 40 deoxyribonucleic acid. J. Virology 6: 788-794, 1970.

3) Levin, M.J., Crumpacker, C.S., Lewis, A.M., Jr., Oxman, M.N., Henry, P.H., and Rowe, W.P.: Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. II. Relationship of adenovirus 2 deoxyribonucleic acid and simian virus 40 deoxyribonucleic acid in the Ad2+ND1 genome. J. Virology 7, 343-351, 1971.

4) Crumpacker, C.S., Henry, P.H., Kakafuda, T., Rowe, W.P., Levin, M.J., and

Lewis, A.M., Jr.: Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. III. Base composition, molecular weight, and conformation of the Ad2 + ND1 genome. J. Virology 7: 352-358, 1971.

5) Henry, P.H., Levin, M.J., and Levine, A.S.: Composition and metabolism of lymphocytes and plasma cells. Chapters 97 and 98 in Principals of Hematology, by Williams, W., Beutler, E., Craddock, C., Erslev, A., and Rundles, R. Blakiston, McGraw-Hill, publishers, 1971.

6) Oxman, M.N., Levine, A.S., Crumpacker, C.S., Levin, M.J., Henry, P.H., and Lewis, A.M., Jr.: Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. IV. Characterization of the simian virus 40 RNA species induced by wild-type simian virus 40 and by the nondefective hybrid virus, Ad2 + ND1. J. Virology (in press).

7) Levine, A.S., Eliot, H.M., Henry, P.H., and Oxman, M.N.: Hybridizable RNA: New species in contact-inhibited as well as transformed hamster cell lines. Science (in press).

Serial No. NCI-3416
1. Medicine Branch
2. Leukemia Service
3. Bethesda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: 1) Infectious Complications of Malignancy: Natural History, Detection, and Therapy

2) The Role of Protected Environments in Cancer Chemotherapy

Previous Serial Number: Same

Principal Investigator: A.S. Levine, M.D.

Other Investigators: Allan Schreiber, M.D., Stuart Siegel, M.D., Edward S. Henderson, M.D., John Bennett, M.D. (NIAID,LCI), D. Henson, M.D. (NINDS,PR), Paul Carbone, M.D., and Seymour Perry, M.D.

Cooperating Units: Acute Leukemia Service
HTCB
LCI, NIAID
Hazelton Labs, TRW Inc.
Litton Industries, Inc.
Infectious Laboratory Service, Clin. Path., CC
Nursing Service, CC
Central Sterile Supply, CC
Pharmaceutical Development Service, CC
Dietetics Service, CC
Environmental Sanitation, CC
Environmental Services, CC
NINDS, N, PR

Man Years:

Total: 7
Professional: 4*
Others: 2*

* Does not include affiliated nursing and other professional care

Project Description:

Objectives:

1) To evaluate the utility and liabilities, separately and together, of various procedures designed to reduce the incidence of severe infection

in patients with acute leukemia receiving induction chemotherapy; to evolve prophylactic techniques and equipment with greater utility than presently available.

2) To improve methods by which occult infectious agents may be detected and eradicated, e.g. latent herpes viruses, L-forms of bacteria, fastidious anaerobic bacteria, mycoplasma, and fungi.

3) To evaluate the human germ-free state, as it occurs in patients with leukemia, and to learn what is the influence of this state on normal physiologic and metabolic processes, and on the metabolism of cancer chemotherapeutic agents.

4) To improve our understanding of the natural history of unusual opportunistic infections, seen primarily in compromised hosts.

Methods Employed

I. Protected Environment Study: Patients with acute leukemia, in the first or second relapse, between 15 and 60 years of age, are randomly and concurrently assigned to receive induction chemotherapy under one of three conditions:

Group I: Patients receive antibacterial baths, sprays, and other modes of topical antisepsis and antibiosis, consume a diet low in bacteria, are confined within a laminar air flow isolater, and are given oral antibiotics (non-absorbable).

Group II: Patients are given the same oral non-absorbable antibiotic regime as patients in Group I, but are otherwise treated conventionally.

Group III: These patients are maintained in the usual ward setting, with no antimicrobial prophylaxis (control group).

Quantitative evaluation of the efficacy of prophylactic techniques is based upon comprehensive sampling of the patients and their environment for aerobic and anaerobic bacteria, fungi, mycoplasma, and viruses. Routine and special culture procedures are employed, as are serologic assays. Criteria for the diagnosis of infection are based on autopsy or biopsy results, cultures, X-rays, and clinical impression (in consultation with Dr. John Bennett, LCI, NIAID). Results are subjected to multiple statistical tests. In vivo and in vitro investigation of possible toxicity from prophylactic techniques is conducted.

II. Studies on Unusual Opportunistic Infections: Routine and special techniques are applied to the isolation of occult agents, immunoflourescent and other serologic assays, and animal innoculation. Such laboratory procedures are correlated with morphologic and cultural results gathered from pathologic specimens.

Major Findings:

I. Protected Environment Study

A) Efficacy of prophylactic components: The gentamycin, vanomycin, and nystatin regime continues to be extremely efficient in reducing the growth of aerobic and anaerobic bacteria in the stool. Between 90 and 100% of all such isolates are suppressed. However, it is likely that these organisms are not entirely eliminated from the gut since patients repopulate with serotypically identical organisms when the oral antibiotics are discontinued. The regime continues to be relatively ineffective in suppressing the growth of gut fungi, with less than 40% of isolates inhibited. However, there has been no increase in the incidence of disseminated fungal infection in the antibiotic-treated patients. No untoward toxicity from the regime has been detected nor has the emergence of organisms resistant to the three antibiotics been noted. In vitro studies have been completed which suggest that should the oral antibiotics be absorbed in significant concentration, they could suppress bone marrow regeneration; however, in vivo absorption has not been significant.

We continue to observe that the same stool culture results obtain whether the patient is given a regular hospital diet or one low in bacteria, so long as the oral antibiotic regime is not a variable.

Regimes for skin antibiosis also appear to be highly effective, with more than 85% of skin samples remaining virtually sterile. However, results with orificial antisepsis continue to be disappointing, with more than half the specimens (nasopharynx, etc.) containing significant numbers of aerobes, anaerobes, and fungi. It has been appreciated in the past year that almost every patient studied has at least one body site in which organisms persist despite an intensive antibiotic and antiseptic regime. Since such sites are often the nidus for disseminated infection, much effort has been spent in identifying such sites and creating a unique eradication regime for each patient.

Four semi-portable laminar air flow rooms have now been installed; the more recent installations include plumbing facilities with advanced concepts of filtration and vacuum suction, thus enhancing sterility and reducing aerosolization of microorganisms. Results to date confirm our previous experience with these units. Fewer than 4 organisms/ 1000 ft^3 are recovered from the air, as contrasted with 10,000/ ft^3 in a conventional hospital room. More than 90% of floor, surface, and water samples are sterile. Of interest is the observation that patients often shed increasing numbers of organisms into the room when site colonization increases; the shedding phenomenon appears to be a prelude to frank dissemination.

B) Results of the controlled study: Improvements were made in the design of the study during the past year so as to improve the accuracy of randomization and to ensure greater comparability between the three

arms of the study. These improvements should allow greater statistical significance with small sample sizes. The data to date follows.

1) Number of induction trials completed: Group I, 16; II, 36; III, 26. Seventy-five percent of these trials were primary, following initial diagnosis, and 75% were in patients with AML (all 3 groups). The distribution of sex, age, and days spent at each granulocyte level were comparable for the 3 groups, as was the regime and total dosage of cancer chemotherapy.

2) Severe infections during induction chemotherapy: Such episodes occurred in 25% of Group I patients, 36% of II, and 46% of III. The trend is encouraging, although the present sample size does not permit a probability value. However, dramatic and statistically significant changes in the incidence of severe pseudomonas infections were noted. Such infections occurred in 27% of Group III patients (controls) but only 6% of I and II. This observation is of particular interest in view of the known lethality of pseudomonas.

3) Infectious deaths: Of great importance was the finding that no infectious deaths occurred during induction chemotherapy of Group I patients out of 16 consecutive trials. Twenty-two percent of Group II and 27% of Group III patients died with severe infection as a primary cause of mortality. These differences have a p value of <0.05. Most infectious deaths in III were caused by pseudomonas; in II, with the same low percentage of pseudomonas as in I, fatal infections were caused by a variety of organisms other than pseudomonas. These infections could usually be traced to a site other than the gut; it is probably that control patients (III) would have had a similar variety of infections had they not died of supervening pseudomonas infections. These observations demonstrate the efficacy of oral antibiotics, as the sole prophylactic technique, in reducing infection caused by enteric organisms; at the same time, they suggest that organisms in other sites will ultimately become apparent once the gut flora is reduced. It is in sterilizing these non-gut sites that the full regime offered to Group I patients has its greatest utility.

4) Days with severe infection and episodes of severe infection as functions of the granulocyte level: 45% of patient days were spent with severe infection in the control group (III), with granulocytes less than $100/mm^3$; also at this granulocyte level, there were 6 episodes of severe infection per 100 patient days on the study. These results indicate that the incidence of severe infection in leukemics at this institution is somewhat less than had been reported in earlier studies from this and other institutions, thus emphasizing the need for concurrent,

controlled studies. As anticipated, the incidence of severe infection was directly related to granulocyte level in all 3 groups. However, at granulocyte levels less than 100, only 1/3 as many days were spent with severe infection by Group I and II patients, as by controls, and only 1/3 as many episodes of infection were found in I and II. These data were statistically significant. At higher granulocyte levels, similar, but less significant, trends were noted.

5) Time until onset of first infection: There was a statistically significant prolongation of the mean number of days on study until the first infection occurred in both Group I and II patients. This period was 5-6 weeks, as opposed to 2-3 weeks in controls. These results indicate that "time can be bought" both by the oral antibiotics alone and by the full regime, sufficient to administer an entire course of chemotherapy.

6) Results of induction chemotherapy: Complete remission was achieved in 25% of Group I patients, 39% of II, and 31% of III. Despite the low remission rate in I, 12 out 15 patients survived for more than 100 days following their entry in the study, as contrasted with 25/35 in II and 13/24 in III. One may conclude that survival is significantly augmented in I and somewhat augmented in II, due to a reduction in infectious deaths. With better survival, it is possible that patients whose tumors initially resist chemotherapy can be given subsequent, successful induction with differing chemotherapy.

Conclusion: Highly encouraging results have been obtained with the use of a comprehensive prophylactic regime, including the use of laminar air flow isolation. These results have not been reflected in increased remission rates (it should be noted that protocol design required drug doses to be similar for all groups) but have certainly augmented survival, such that subsequent induction attempts would become possible. Benefits have also been obtained from the use of oral antibiotics alone, although they are considerably more limited than those obtained with the full protection program.

II. Transmission of Toxoplasmosis: 6 patients with acute lymphocytic leukemia developed disseminated toxoplasmosis following multiple leukocyte transfusions from donors with chronic myelocytic leukemia. Such transfusions were administered because of granulocytopenia complicated by bacterial infection. Longitudinal serological assays of donors and recipients strongly suggested that concentrated leukocyte transfusions might be one mode of transmission of this protozoan. Such a conclusion is supported by the observation that the organism is able to parasitize all nucleated cells in various animal models, and that it is often seen in leukocytic infiltrates on pathological section of diseased human tissue. The recomendation was made that potential blood donors to patients with altered defenses be carefully screened for high levels of anti-toxoplasma

IgG, and for any level of anti-toxoplasma IgM.

III. Longitudinal Cytomegalovirus Study: 87 leukemic children were followed for one year with serial assays of virus excretion and antibody levels; correlations were made with clinical syndromes consistent with CMV and with pathological proof of dissemination. Such a longitudinal study had not been previously undertaken. Twenty-three children excreted the virus at some point during the year, a percentage comparable to that in the normal age-matched population. However, most of these patients had low or absent antibody to CMV during the year; only 6 virus excretors demonstrated a 4-fold rise in antibody at some point, and 4 additional patients had 4-fold rises in antibody without ever excreting the virus. Pathological and cultural evidence of disseminated infection was found only in children who had had 4-fold antibody rises, whether or not they had excreted the virus. We concluded that the best proof of CMV dissemination is a demonstration of significantly rising antibody titers during a clinical syndrome consistent with CMV, whether or not virus is excreted; virus excretion without rising antibody titers does not indicate dissemination. The infection rate in leukemics is not different than in the age-matched normal population, but the incidence of widespread dissemination is greater. These results appear to illuminate our understanding of a poorly characterized opportunistic infection.

IV. Diagnosis of Systemic Candidiasis: A prospective study of anti-candida antibodies in patients with acute leukemia was completed. Correlations were made between cultural and pathological proof of invasive disease, and serological evidence of infection. The utility and inadequacy of the various serological procedures available was evaluated.

Proposed Course

1) The controlled, randomized study of protected environments will continue until such time as we can demonstrate with statistical significance, in all parameters, the ultimate utility of the various prophylactic components. It should be possible to reach this goal within 6-12 months. If the present trends continue, the oral antibiotic arm (Group II) will be discontinued, since it appears less effective than the full regime and involves a continuing large innoculum of organisms concurrent with the selection pressures inherent in the chronic use of antibiotics. However, the results to date seem to warrant a recommendation that institutions not in a position to install laminar flow rooms might use oral antibiotics alone in selected patients. Ultimately, in this institution, the various modes of therapy used in Group I should be afforded to all granulocytopenic patients, assuming maintenance of the present trends in the controlled study.

2) The activities proper to patient protection research will be expanded with the addition of a Ph.D. microbiologist who will aid in these developments: detection and eradication of conventional and opportunistic pathogens, including agents for which present techniques are inadequate, e.g. herpes viruses and fungi. Further, we shall undertake drug trials of newer

anti-viral and anti-fungal agents in the treatment of established infection. Studies will also involve manipulative procedures that might render deleterious organisms harmless in terms of patient morbidity, but permit more normal physiology than the germ-free state. Such procedures might include ablation and recolonization; the use of phage to alter the genotype of lethal organisms; and the use of selective procedures to allow the overgrowth of innocuous organisms. The basic surveillance sampling necessary to implement the patient protection program will be conducted by Hazelton Labs, and the microbiologist assigned to the Leukemia Service will be responsible for quality-control of the contract work. With expansion of our facilities and personnel, it should be possible to investigate alterations in metabolism, immune competence, and chemotherapeutic toxicity which may result from the human germ-free state.

3) Exploration of infections caused by opportunistic organisms in compromised hosts will continue, involving all patients on the Leukemia Service as well as those in the patient protection program. This work will proceed with the view that these infections are virtually unique, in their frequency and degree of dissemination, to patients with malignancies. Consequently, opportunities exist for a better understanding of the biology of these agents than are available to conventional infectious disease sections of general medical services. Since the proposed scope of these investigations is exceedingly broad, it will be necessary to expand our intramural collaborative efforts, as well as contract research.

Honors and Awards:

Invited Lectures:

1) "Infectious Complications in the Altered Host," Arthur S. Levine, M.D., Divisions of Infectious Disease, Departments of Pediatrics and Medicine, Harvard Medical School, Children's Hospital Medical Center, Boston, April 4, 1971.

2) "Gnotobiotic Techniques in the Treatment of Hematopoietic Malignancies," Edward S. Henderson, M.D., Chairman. XIII International Congress of Hematology, Munich, Germany, August, 1970.

3) "Ecology of Laminar Air Flow Rooms as Contrasted With Normal Hospital Rooms", Miss Janet Hauser. XIII International Congress of Hematology, Munich, Germany, August, 1970.

4) "Protected Environments in Cancer Chemotherapy", Edward S. Henderson, Ontario Cancer Research Council, Toronto, Canada, 1970.

5) "The Role of Protected Environments in the Treatment of Acute Leukemia", Edward S. Henderson, M.D., Johns Hopkins Hospital, Baltimore, Md., 1970.

Publications:

1) Preisler, H.D., Goldstein, I.R., and Henderson, E.S.: Gastrointestinal sterilization in the treatment of patients with acute leukemia. Cancer 26: 1076-1081, 1970.

2) Henderson, E.S., Preisler, H.D., and Goldstein, I.M.: Studies of components of patient protection programs: Nonabsorbable antibiotics and low bacterial diets. Recent Results in Cancer Research 29: 24-30, 1970. Springer-Verlag, Berlin.

3) Perry, S., and Penland, W.: The portable laminar flow isolator: New unit for patient protection in a germ-free environment. Ibid., 34-40.

4) Siegel, S., Lunde, M., Gelderman, A., Halterman, R., Brown, J., Levine, A., and Graw, R.: Transmission of toxoplasmosis by leukocyte transfusions. Blood (in press).

5) Roth, J., Siegel, S., Levine, A., and Berard, C.: Fatal recurrent toxoplasmosis in a patient initially infected via a leukocyte transfusion. Amer. J. of Clin. Path., (in press).

6) Preisler, H.D., Bruckner, H., and Henderson, E.S.: Reduced production of spleen colonies and granulocytes in antibiotic-treated irradiated mice transplanted with syngeneic bone marrow. Radiation Research (in press).

7) Preisler, H.D., Hasenclever, H., and Henderson, E.S.: Anticandida antibodies in patients with acute leukemia: A prospective study. Amer. J. Med., (in press).

Serial No. NCI-3419
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Transplantation and Immunotherapy

Previous Serial Number: Same

Principal Investigators: Brigid Leventhal, M.D., Robert Graw, M.D., Ronald Yankee, M.D., G. Nicholas Rogentine, M.D., Edward Henderson, M.D., Roger Halterman, M.D., and Geoffrey Herzig, M.D.

Other Investigators: Jacqueline Whang-Peng, M.D., Mary McGinniss, Gerhard Krueger, M.D., Ronald Herberman, M.D., and Eugene Rosenberg, M.D.

Cooperating Units: Human Tumor Cell Biology Branch, NCI
Immunology Branch, NCI
Anatomical Pathology Branch, NCI
Hematology Service, Clinical Pathology Dept., Clinical Center, NIH
Blood Bank, Clinical Center, NIH
Anesthesiology Service
National Institute of Allergy and Infectious Disease
National Institute of Arthritis and Metabolic Disease
University of California at Los Angeles, Department of Surgery

Man Years:

Total: 11
Professional: 6
Other: 5

Project Description:

Objectives

1) To assess the optimal methods of obtaining allogeneic and isogeneic bone marrow grafts in human leukemia patients.

2) To assess the use of marrow stem cell grafting as an adjunctive measure in leukemia therapy.

3) To study cellular recognition of transplantation antigens in vitro.

4) To investigate the existence of immunity against tumor systems by delayed hypersensitivity and in vitro lymphocyte transformation.

Major Findings:

A. Marrow transplantation: We have continued an active program in bone marrow transplantation in the hope the marrow transplantation will prove to be an adjunct to chemotherapy and possibly even have an antileukemic effect in itself. Since the seven grafts attempted in six recipients cited in last year's report, we have performed four additional marrow grafts in patients with acute leukemia. All were performed using the four day cyclophosphamide regimen at 45 mg/kg/day as previously described for immunosuppression of the marrow recipient, and all donor recipients pairs were HL-A matched with genotypically identical siblings.

The first patient was one of the patients in our 1970 series who received a marrow graft from an HL-A identical sister. Leukemia recurred in the marrow on day 123. She was then treated with cyclophosphamide without marrow infusion and 20 days following the last dose of drug, a hypocellular bone marrow without ALL returned. During this period, she required regular platelet support from her marrow donor because of marrow hypoplasia; 112 days later, leukemia recurred again in the marrow. It was decided to perform a second marrow transplant from the same donor 412 days following the first. During the pre-transplant evaluation, it was found that the donor immunoglobulin type has persisted, and donor red cell antigen E had reappeared in the recipient after therapy of the leukemia relapse with cytoxan. The patient had return of normal bone marrow cellularity with complete disappearance of the leukemia after the second marrow infusion. She has apparently been a bone marrow chimera for over one year.

The second patient had acute lymphocytic leukemia refractory to chemotherapy. Before cytoxan was given, she had received 41 units of platelet concentrates from her HL-A matched brother who subsequently donated bone marrow. In animal systems, there is evidence that grafts will be rejected in recipients who have been previously transfused. In spite of the possibility for specific prior sensitization of the recipient, there was evidence of marrow engraftment as determined by the appearance of cells with donor karyotype in the bone marrow and peripheral blood on day 22 after marrow infusion. Sixty-seven days following transplantation, there was full return of ALL in cells of recipient karyotype.

The third patient was a 6-year-old white male with ALL of approximately two years duration. He achieved remission with asparaginase and was then given cyclophosphamide followed by bone marrow from his HL-A identical brother. No methotrexate was administered post transplant since it was unclear from earlier cases whether it had influenced either the incidence or severity of graft vs. host reaction (GVHR). On day 31, he developed a generalized exfoliative skin eruption with gastrointestinal and hepatic malfunction. Death occurred from Aspergillus pneumonia on day 69. Histologic sections

at post mortem were consistent with GVHR.

The fourth patient was a 27-year-old woman with acute myelocytic leukemia resistant to therapy after seven months. She had a severe skin rash which histopathologically was thought to be GVHR within one week following marrow infusion. There was no apparent recovery of normal marrow function after cyclophosphamide. Marrow by day 16 post-transplant was extensively infiltrated with leukemia. She survived her GVHR and died 31 days post-transplant of sepsis and bone marrow aplasia complicating further attempts at chemotherapy of her leukemia.

Laboratory support for these studies has included an active program for HL-A typing under contract with Microbiological Associates in Bethesda and a mixed-leukocyte culture program at the National Institutes of Health. All patients have been extensively investigated. The immune competence of these bone marrow chimeras has been of particular interest. Patients were studied for cellular reactivity in vitro, skin test reactivity, and antibody response to infection and immunization in 4 patients who represented a spectrum of GVHR. We conclude that depressed immunologic capacity might be proportional to the severity of GVHR in the chimeras. Cellular immunity appeared more depressed than humoral. Despite the apparent defect in cellular immune function, three of the four patients survived viral infections while in the chimeric state. Two had cytomegalovirus infection and one herpes zoster. This is of interest in view of the other reports of fatal infections in apparently stable human marrow chimeras.

Conclusion:

1. Fatal GVHR can occur despite HL-A identity. Despite complete tissue matching in the HL-A system, as currently defined by serotyping and mixed leukocyte culture assay, we have seen a spectrum of GVHR in these 10 patients including 2 deaths. It is obvious that post-transplant immunosuppression is necessary during marrow grafting with cyclophosphamide.

2. Grafts can be successfully performed even when the recipient has received prior blood products (platelets) from the marrow donor. Animal evidence would suggest that marrow grafting is unlikely to be successful if the recipients have received transfusions. All of our patients had had many units of transfused blood from random donors, and case 2 in this report had received multiple units of platelets from the prospective marrow donor. It is true that this patient may have been immunosuppressed by her antileukemia therapy at the time of transfusion so that sensitization did not occur. However, we find these results encouraging in that successful grafting may be deferred until drug trials requiring transfusion support have been completed.

3. An established marrow graft persisted throughout a course of

cyclophosphamide which eliminated recurrent acute lymphocytic leukemia from recipient marrow. This indicates that in some cases allogeneic marrow grafting may provide a most useful adjunct to myelotoxic leukemia chemotherapy.

4. The immune competence of human marrow chimeras is altered perhaps proportionate to the severity to the GVHR.

5. There is no evidence to date for any specific anti-leukemia effect of the marrow graft itself.

B. Other studies defining the nature of HL-A antigens: The mixed leukocyte culture has provided a tool to investigate reactivity to HL-A antigens. We had the opportunity to serially study six patients sensitized with intradermal injections of purified HL-A antigen prepared from the human tissue culture cell lines IM-1 and RAJI. Soluble HL-A antigens have been reported to regularly produce substantial lymphocyte transformation with reactivity that was in general greater than that of comparable mixed leukocyte cultures in non-sensitized individuals. Our results contrasted with these findings in several respects. First, there was no response in five of six patients prior to sensitization. Second, responses were seen after sensitization in five patients but these were, in general, low grade and transient. One would have to conclude from our studies that antigen presented to the lymphocyte in a soluble form, although active, is less stimulatory to these lymphocytes than antigen presented to lymphocytes on the intact cell surface. We have also had an opportunity to extend our study of antibody and its effects in mixed leukocyte culture. Lymphocytes from five mothers were washed and tested against mitomycin treated cells from 13 of their children. With the addition of maternal plasma reactivity was abolished in nine cases and diminished in three. The decrease in MLC reactivity could be shown to be a function of diminished stimulating capacity of cells bearing specific antigens. These same plasmas were shown by immunofluorescence to contain IgG antibodies which coated human lymphoid tissue culture cells of known HL-A specificities. It is concluded that an antibody is present in maternal plasma which coats stimulating cells and decreases their capacity to stimulate in MLC.

Conclusion:

The mixed leukocyte culture is an extremely valuable tool to investigate both the specificity and the mechanism of response to HL-A, the major histocompatibility antigen system in man.

C. Studies for detection of tumor antigens on cells of patients with acute leukemia: The existence of specific tumor antigen on leukemia cells can only be investigated if these malignant cells are available for study at the time the patient has enough circulating normal cells to mount a detectable immune response. When a new patient is admitted to the Leukemia Service, blast cells are harvested from bone marrow and/or peripheral blood.

These cells are diluted with medium and 10% DMSO and then frozen at 1-3°C/minute and stored in liquid N_2 (less than −130°C). To date, cells have been harvested and stored on 70 patients. The patient is then treated at various intervals, cells are thawed and normal peripheral blood lymphocytes are drawn from the patient and tested for reactivity against the autologous blasts in the cellular cytotoxicity assay described by Canty and Wunderlich (JNCI 45: 761, 1970) and in the mixed leukocyte culture. At the same time membrane preparations of relapse and remission cells are used to test intradermal reactivity in the patient. To date over 100 assays have been performed. CCA was positive in 3/9 acute myelocytic leukemia and 4/9 acute lymphocytic leukemia patients; MLC was positive in 9/14 acute myelocytic leukemia and 5/11 acute lymphocytic leukemia patients; and skin tests were positive in 8/11 acute myelocytic leukemia and 7/10 acute lymphocytic leukemia patients. There was no apparent correlation between in vitro tests and clinical status. CCA was positive in 5/13 remission and 4/7 relapse patients. There was a suggestion that skin tests were positive more often in remission with 13/16 positives compared with 2/5 patients positive in relapse. Two of the three negatives and only one of 13 positives relapsed within a month of the skin test. A similar possible correlation of positive skin tests with remissions status has been seen in Burkitt's lymphoma. In vitro reactivity appeared consistent throughout the observation period of each patient. On repeat testing in MLC 3 positives remained positive, 4 negatives remained negative and only one patient with a positive test converted to negative. Eight patients were tested in MLC against remission marrow as well as relapse. Five reacted to their relapse marrows while only one of these also reacted to the remission marrow.

Conclusion:

The majority of leukemia patients shows some evidence of immunologic reactivity against their own malignant cells. In all, 10 of 14 patients with acute myelocytic leukemia and 8 of 11 with acute lymphocytic leukemia reacted to their own leukemia cells in at least one of the three assays. We plan now to use these assays to follow the reactivity of patients against tumor in the course of chemotherapy and immunotherapy protocols.

D. Twin study: Twin pairs in which one twin has leukemia have been solicited. Six pairs of twins have been studied with the cellular cytotoxicity assay, MLC, and skin testing. All patients were in remission when tested. To date there has been no evidence of reactivity between cells from the well versus the leukemic twin in remission in MLC. In the cellular cytotoxicity assay there was evidence of reactivity of well twin, family members and unrelated donors against remission cells of leukemia twin.

Conclusion:

Identical twins of patients with leukemia are ideal controls to use

in studies of the immunologic responsiveness of patients to autologous tumor. There is a suggestion that family members are reactive to remission cells of leukemia patients.

Significance:

Much information has been gained about the techniques of bone marrow grafting in man. We feel that cyclophosphamide pretreatment of the recipient guarantees a high percentage of graft takes (10/11). Pre-exposure of the recipient, even to blood products from the specific individual who is later used for a marrow donor, will not invariably prevent graft acceptance. Practical use of bone marrow transplantation will require an effective regimen of post graft immunosuppression and further study and understanding of the immune competence of the bone marrow chimeras. As we gather more information on these points we will be able to graft patients at an earlier stage in their disease. There is no evidence to date for any specific antileukemia effect of the marrow graft itself.

Hoping to use HL-A as a model for possible tumor specific transplantation antigens in man, we have done some basic studies with purified HL-A antigen and anti-HL-A antibodies. It is apparent that antigen presented in a purified form is less stimulatory to the lymphocyte than that presented on the surface of the whole cell. It is also clear that specific antibodies can block the response to these antigens presumably by coating the target cell. The mixed leukocyte culture appears to be a good tool with which to investigate both the specificity and the mechanism of response to HL-A in man.

The majority of patients with acute leukemia (18/25) shows some reactivity against their own blast cells in one of three assays; cellular cytotoxicity, mixed leukocyte culture, and skin test. We feel that we are now in a good position to follow immune reactivity of patient against autologous tumor during projected immunotherapy protocols. These stored blasts are also acting as a resource for other laboratories who are interested in studying their biochemistry, kinetics, etc.

Proposed course of the project:

We will continue to investigate more effective ways of treating GVHR in allogeneic marrow grafts. We are also starting a program of storing autologous marrow on a patient that might be reinfused after comparable chemotherapy and used as a control in the study of the antileukemia effect of the allogeneic graft.

The *in vitro* work with purified HL-A antigen will continue. This is of interest in itself and as a model for possible tumor antigens. We are currently attempting to fractionate plasma to determine which type of antibody is responsible for the MLC inhibition. We are also investigating the best form of HL-A antigen to stimulate the lymphocyte in hopes of eventually being able to use antigen to induce either increased sensitivity or tolerance in a controlled fashion.

The response of leukemia patients to their own tumor is most exciting and we plan to follow this during therapy with allogeneic leukemia cells as well as with chemotherapy and nonspecific immune adjuvants such as BCG, MER, or rifampicin. We also plan to perform allogeneic testing of patients with categorically similar leukemias to determine whether or not cross reactivity can be detected. Reactivity of family members against a leukemic antigen on patients cells is also under active investigation.

Honors and Awards:

Ronald A. Yankee:

1) Bone marrow transplantation, presented at the meeting of the American Association of Blood Banks, San Francisco, October, 1970.

Edward S. Henderson:

1) Bone marrow transplantation in acute leukemia, presented at the International Society of Hematology, XIII Congress, Munich, August, 1970.

2) Immunotherapy of acute leukemia, presented at the Hahnemann Medical School Symposium, Philadelphia, November, 1970.

Publications:

1) Graw, R.G., Brown, J.A., Yankee, R.A., Leventhal, B.G., Whang-Peng, J., Rogentine, G.N., and Henderson, E.S.: Transplantation of HL-A identical allogeneic bone marrow to a patient with acute lymphocytic leukemia. Blood 36: 736, 1970.

2) Graw, R.G., Buckner, D., Whang-Peng, J., Leventhal, B.G., Kruger, G., Berard, C., and Henderson, E.S.: Complications of bone marrow transplantation: Graft versus host disease resulting from chronic myelogenous leukemia leukocyte transfusion. Lancet 2: 338-341, 1970.

3) Graw, R.G., Jr., Rogentine, G.N., Yankee, R.A., Leventhal, B.G., Whang-Peng, J., Herzig, G.P., Halterman, R.H., Kruger, G., Berard, C., and Henderson, E.S.: Graft versus host reaction complicating HL-A matched bone marrow transplantation. Lancet 2: 1053, 1970.

4) Kruger, G., Berard, C.W., Elias, P.M., Graw, R.G., Jr., Rogentine, G.N., Leventhal, B.G., Yankee, R.A., Herzig, G.P., Halterman, R.H., and Henderson, E.S.: Morphology of graft-versus-host reaction in HL-A matched bone marrow transplantation and its differential diagnosis. Experimental Hematol., (in press).

5) Halterman, R.H., Leventhal, B.G., Graw, R.G., Jr., Yankee, R.A., Rogentine, G.N., Herzig, G.P., Henderson, E.S.: Immunocompetence of patients following HL-A matched bone marrow transplantation. Experimental Hematol. (in press).

6) Graw, R.G., Jr., Yankee, R.A., Leventhal, B.G., Rogentine, G.N., Herzig, G.P., Halterman, R.H., Kruger, G.R., Berard, D.W., Henderson, E.S.: Graft versus host disease complicating HL-A matched bone marrow transplantation. Experimental Hematol. (in press).

7) Graw, R.G., Jr., and Santos, G.W.: Bone marrow transplantation in patients with leukemia. Incidence and severity of GVHR in successful HL-A matched transplants. Transplantation 11: 197, 1971.

8) Leventhal, B.G., Buell, D.N., Terasaki, P., Yankee, R. and Rogentine, G.N. Jr.,: The mixed leukocyte culture: Effect of maternal plasma. In Harris, J. (Ed.): Proceedings of the Fifth Annual Leukocyte Culture Conference, Academic Press, New York, p. 473,1970.

9) Graw, R.G.,Jr., Leventhal, B.G., Yankee, R.A., Rogentine, G.N., Whang-Peng, J., Herzig, G.P., Halterman, R.H., and Henderson, E.S.: HL-A and mixed leukocyte culture matched allogeneic bone marrow transplantation in patients with acute leukemia. Transplantation Proceedings (in press).

10) Leventhal, B.G., Mann, D.L., and Rogentine, G.N., Jr.: Sensitization to water soluble HL-A antigens. Transplantation Proceedings (in press).

11) Graw, R.G.,Jr., Brown, J.A., Yankee, R.A., Widerlite, L.W., Leventhal, B.G., Whang-Peng, J., Rogentine, G.N., and Henderson, E.S.: Transplantation of HL-A and MLC identical bone marrow to patients with acute lymphocytic leukemia. Experimental Hematol. 20: 35, 1970.

12) Henderson, E.S., and Leventhal, B.G.: Immunotherapy of leukemia. Proceedings of the Hahnemann Hospital Symposium (in press).

Serial No. NCI-3403
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Clinical Trials and Miscellaneous Clinical Investigations

Previous Serial Number: Same

Principle Investigators: Vincent T. DeVita, George P. Canellos, Robert C. Young

Other Investigators: Charles Bagley, Arthur Serpick, Paul P. Carbone, Oleg Selawry, and Ralph Johnson

Cooperating Units: VA Oncology Unit, NCI
Radiation Branch, NCI

Man Years:

Total:	2.5
Professional:	2.0
Other:	0.5

Project Description:

Clinical trials on the Solid Tumor Service involve two major areas:

1. New antitumor drugs developed through the NCI program are evaluated for toxicity and therapeutic benefit in patients with metastatic cancer (Phase I Studies).

2. Drug sensitive tumors; that is, tumors for which several drugs are known to be effective; are studied in an attempt to exploit current information derived in sensitive animal tumors to develop improved treatment programs.

The Solid Tumor Service has a disease oriented program. The following drug sensitive tumors are under study:

1. Ovarian carcinoma
2. Hodgkin's disease
3. Lymphosarcoma
4. Reticulum cell sarcoma
5. Carcinoma of the breast
6. Chronic myelocytic leukemia

Phase I studies are conducted on patients with the above tumors who fail on the primary treatment programs, to aid in the identification of additional drugs for these patients, and in patients with tumors known to be resistant to all currently availabe drugs (malignant melanoma, osteogenic sarcoma, etc.). Additional clinical investigations are conducted in support of the above clinical trials, some of which are reviewed under projects entitled Kinetics of Cellular Proliferation, Immunologic Aspects of Cancer, and Physiologic Disposition and Metabolism of Drugs. A separate project describing the studies in chronic myelocytic leukemia should also be referred to.

A. Disease Oriented Studies

1. Ovarian Carcinoma

Carcinoma of the ovary is one of the most common tumors of women, affecting 17,000 women each year. Eleven thousand of these patients die each year, because early detection is difficult, and most present with advanced disease (Stage III or IV).

It is the opinion of our Staff that this common tumor is now ready for an intensive study. Overall it appears that we are in the same position now with ovarian cancer as we were with the lymphomas in 1963. Although the tumor is radiosensitive, X-irradiation suffers from the same defect as surgery, in these patients, in not being able to include all tumor in the radiation fields. This fact probably accounts for the fact that, in the last decade, radiation therapy added to surgery has not improved the survival of patients with any stage of the disease. The overall 5-year survival remains 25 percent. The best results have been reported, in the literature, from the addition of drugs to the radiation program and the question has been raised as to the efficacy of the use of radiation at all in advanced stages, since it effectively limits the proper use of drugs. In fact, not a single controlled trial comparing drugs alone to X-irradiation alone in equivalent stages has ever been reported.

There is some suggestive evidence from long-term followup of patients with advanced disease treated with alkylating agents alone, after surgery, that cures are obtained, if one accepts 9 years free of disease as cure (this author does).

Several other interesting features of this tumor should be pointed out:

a. Patients generally have ascites and often die as a result of disease in the abdominal cavity. These cells are available for cell kinetic analysis and a host of other studies.

b. These measurements should be relevant, because the measurements are on cells in a site that leads to the death of the host.

c. Estimates of cell kill per dose can be made with ease to guide therapy.

d. Distribution of drug to the tumor site can be readily studied.

All these facts have led to a high degree of excitement for the study of this common, and commonly lethal, tumor. Accordingly, a major program on the chemotherapy of adenocarcinoma of the ovary has been begun. A great deal of interest and enthusiasm in the chemotherapy of this tumor has been generated because of the observations that it is responsive to alkylating agents, 5-Flourouracil, methotrexate, and Actinomycin-D, as well as other chemotherapeutic agents. Responses to chemotherapeutic agents predictably result in prolonged survival and useful life in those patients who do respond (20 percent complete responders with alkylating agents). As a consequence, studies have been initiated comparing standard therapy, intermittent oral phenylalanine mustard, with high-dose intermittent intravenous cyclophosphamide therapy. Patients with Stage III and Stage IV disease, not amenable to initial surgical cure, are selected for the study and are randomly allocated by histologic type to one of the two initial groups. At the same time, information as to the response rates of other chemotherapeutic agents will be ascertained, and using information from the initial study, an acceptable combination chemotherapy program for ovarian carcinoma will be instituted. Seven patients have been randomly allocated to this program.

2. Hodgkin's Disease

The combination chemotherapy drug program has reached fruition, and 5-year followup data are now available for analysis. Sixty percent of the entire group is alive at 5 years. If one considers the complete responders (81 percent of the total group), 77 percent are alive at 5 years, and 41 percent are, and have been, continuously free of disease with no treatment after the first 6 months of drug therapy. Since these are patients who presented with very advanced disease, these results are encouraging.

Since 1967, complete responders have been randomly allocated to three different categories: (1) no further treatment, as in the original study; (2) intermittent treatment with the drug combination for an additional 15 months; and (3) intermittent treatment with the new agent BCNU (see below). Enough patients should be allocated to the various arms of this program in the next year to report on the effects of this approach and the duration of complete responses by next year.

Other findings of interest in regard to the use of the drug combination in Hodgkin's disease are:

a. Previously heavily irradiated patients, who recur, can be successfully treated with little difficulty with the combination.

b. A random allocation of patients with Stage IIIB disease between total nodal irradiation and combination chemotherapy proved the combination drug program superior to total nodal X-irradiation.

c. The effects of combination drug treatment on reproductive capacity of males has been studied with interesting results. (See below under Clinical Investigation.)

In the preceding year, many institutions and cooperative groups have been conducting studies using this treatment approach and have confirmed the results reported above.

Preliminary evaluation of the control arm (no drug therapy) indicates that the failure rate is higher than with continued drug maintenance. In the coming year, we propose to abandon this arm of the study in favor of one employing nonspecific immunotherapy (BCG innoculations) to test its efficacy in patients who have achieved a remission.

A summary of our experience with BCNU in patients with far-advanced Hodgkin's disease has been completed. BCNU is a member of a new class of antitumor agents, the nitrosoureas, which have demonstrated activity against several human tumors. BCNU has been used to treat 45 patients with far-advanced, previously treated, Hodgkin's disease; 21 patients (47 percent) achieved a significant remission. Although complete remissions are uncommon, the mean duration of the two complete remissions was 140 weeks, compared to 16.5 weeks for those patients achieving a partial remission. The toxicity of the drug was significant, but tolerable; the major toxicity is a delayed bone marrow suppression which predictably causes a fall in peripheral counts 4 to 6 weeks after a single intravenous dose. Mild toxicity in the form of nausea and vomiting, and burning at the intravenous site, were also noted. BCNU has activity against Hodgkin's disease even when the disease is resistant to standard chemotherapeutic agents, and it appears not to be cross-resistant with the alkylating agents. BCNU offers physicans another useful agent in the control of Hodgkin's disease.

Other agents now undergoing Phase I trials are being evaluated in a Phase II fashion against Hodgkin's disease; specifically Bleomycin and procarbazine (see below).

3. Lymphosarcoma

Since 1967 the Solid Tumor Service has been involved in a randomized, controlled clinical trial of optimal chemotherapy versus optimal radiotherapy in patients with Stages III and IV (nodes plus marrow only) lymphosarcoma.

The drug approach has involved the use of a three-drug combination, since

previous controlled studies have shown that the ability of drug combinations to induce complete remissions is superior to single agents. Other studies have shown that complete responders live longer than nonresponders. Too few cases have accumulated to make serious comparisons possible, but in equivalent stages, both programs appear to be doing equally well. The results of the "optimal" treatment programs in patients with Stage IV disease (nodes plus marrow and other organs) not randomized to X-irradiation were reported for the first time this year.

Thirty-five patients were evaluated; 57 percent achieved a complete remission; most remain in complete remission as of this followup time. Survival of the complete responders was 95 percent at 1 year, and 85 percent at 2 years. Too few cases go beyond that point for accurate analysis. The median duration of complete responses and survival has not been reached, but appears to be superior to any program previously reported or published for patients with advanced lymphosarcoma.

4. Reticulum Cell Sarcoma

Based on the observation that one-third of our patients with reticulum cell sarcoma can be rendered free of disease and remain free of disease up to 5 years after therapy, we have been involved in a controlled clinical trial with the Radiation Branch, NCI, in evaluating drugs versus X-irradiation or drugs plus X-irradiation treatment in patients of equivalent stages. Previous detailed review of the medical literature reveals that X-irradiation alone in the past has been able to "cure" only 10 percent of all patients with this disease. The major reason for their failure is the fact that most patients who appear to have localized disease, in fact, have unrecognized dissemination outside the radiation field. We have embarked on an extensive staging evaluation before therapy to help define the extent of disease (see below). The randomization scheme for reticulum cell sarcoma is as follows:

a. Stage I and II are randomly allocated to X-irradiation alone or chemotherapy followed by X-irradiation.

b. Stage III is treated by chemotherapy, followed by X-irradiation to involved nodes. Too little data has been accumulated to comment on efficacy. It is estimated that the study will take 3 years to evaluate.

5. Carcinoma of the Breast

Carcinoma of the breast is a common malignancy that has demonstrated responsiveness to a varity of therapeutic assaults, including hormones, radiation, and chemotherapy. The number of controlled drug studies, however, have been few, although many patients have been treated. The objects of the present drug studies are two-fold: (1) to explore the

efficacy of adjuvant chemotherapy, administered to Stage II (axillary nodes positive) patients shortly after mastectomy; (2) to explore the use of combinations of effective agents in patients with far-advanced disease; and (3) to test the use of new agents in carcinoma of the breast.

a. Adjuvant Chemotherapy

The use of the double-blind approach has been elected. Phenylalanine mustard (PAM), 0.15 mg/kg/day, for 5 days, every 6 weeks. Patients with Stage II disease only are included on this study. At the present time, six patients are enrolled in the study. One has relapsed on PAM. There has been no serious toxicity from this drug to date.

b. Combination Chemotherapy

The use of multiple agents in cycles of treatment has been elected as an approach to combination treatment. The ideal regimen would allow optimal dosage to be administered for prolonged periods of time. The regimen included 2 weekly doses of 5-Flourouracil and methotrexate (IV) and 14 days of daily oral prednisone and cyclophosphamide. These follow a 2-week recovery period and the cycle is repeated with adjustments of the dose as necessary. Patients with measurable disease refractory to conventional hormone therapy are to be included. Four patients only have been entered on this study to date, and it appears easily tolerable. Two of these have thus far responded well.

c. New Drugs or Combinations

The combination of the drugs BCNU and 5-Flourouracil was explored in seven patients with advanced, untreated breast disease. The goal of this protocol was to explore an observation in the L1210 mouse tumor system, where BCNU was shown to prolong the DNA synthetic period of the cell cycle.

The addition of the antimetabolite, 5-FU, was to increase the potential cell kill in a cell population affected by BCNU. There are examples of increased efficacy of such combinations in animal tumor systems. Only one patient in seven achieved an excellent response to this combination. The major toxicity was severe. This approach was deemed not suitable for extensive trial.

The combination drug programs are controlled by random allocations to the same drugs used singly in sequence.

B. Phase I Studies

1. Achronycin (NSC 403,169)

This compound is an alkaloid derivative isolated and prepared by Eli Lilly Co. for clinical trial. It is derived from the bark of Achronychia Baueri. Interest in this agent is derived from the fact that it is highly active against certain animal tumors which are resistant to other drugs, specifically the C-1498 leukemia, carcinoma 115, and the adenocarcinoma 755 of mice. It was of special interest that the X-5563 plasma cell myeloma of mice was completely inhibited and may indicate that this drug has some potential for human myeloma. Preclinical toxicity and initial clinical trials suggested a safe initial dose at 100 mg/m^2/day (continuous) orally. Eleven patients have been treated, with groups of three at each dose level, and 50 percent escalation of the dose. Aside from gastro-intestinal distress with emesis, meylotoxicity has yet to be seen, at 450 mg/m^2/day x 15 days. No objective antitumor effect has been seen except, of interest, one elderly patients with advanced myeloma did have a drop in the globulin spike of over 1.0 gm%. The study continues.

2. Bleomycin

This new polypeptide antitumor antibiotic is a Japanese compound of uncertain nature which shows marked antitumor activity against certain squamous cancers in Japan. A broad national trial of the drug in the U.S. and the early reports of antilymphoma activity prompted a trial of this agent in advanced lymphomas refractory to all other active compound, including BCNU. The severity of disease and the advanced clinical state precluded any but intensive scheduling. A larger phase I cooperative study with the NCI-VA oncology unit and Roswell Park, using a twice-weekly schedule is also in progress in a variety of solid tumors. A course of 25 mg/m^2/day IV x 5, was administered to eleven consecutive adult lymphoma patients (5 lymphosarcoma, 5 Hodgkin's disease, one reticulum cell sarcoma). Out of this group, two transient, partial responses were noted. In addition, two patients with Hodgkin's disease had a significant, but transient palliation, of systemic febrile symptoms. The significant toxicity includes oral ulcerations, 2/11 patients; thrombopenia, 1/11; acute respiratory distress, 1/11. Induration of the skin, alopecia, fever, and dermatographia have also been common, but not dose-limiting. Patients with diseases other than lymphoma have been entered into the other study employing the intermittent dose schedule in cooperation with the NCI-VA Oncology Unit (see their report). To date, our experience tends to confirm the antitumor activity in embryonal cell carcinomas. Further studies with this compound with lymphoma will include its use in combination with other drugs to exploit its lack of marrow toxicity.

3. NSC 82196, the Imidazole Carboxamide Derivative

This drug, developed in the drug screening program at the NCI, has been in Phase I clinical trial for almost 1 year. Twenty-eight patients have been studied, with doses ranging from 100 mg/m^2 to the current top dose,

5000 mg/m^2 given over a 5-day period. The most severe limiting side effect has been the presence of nausea and vomiting. Leukopenia has not been dose limiting thus far, and has been noted at lower dose levels only in those patients whose marrow has previously been compromised by extensive prior treatment. Thrombocytopenia has been noted in similar patients. Other side effects have not been severe. One minimal response has been noted in a patient with malignant melanoma. It appears that 5000 mg/m^2 given over a 5-day period and repeated cyclically may be close to a top dose. This dose will be tested further in patients with metastatic malignant melanoma. Studies are underway to further examine the immunosuppressive capabilities of this drug, which in a previous study from our laboratory proved superior to many available drugs tested in parallel.

4. Polyinosinic-cytidylic acid (Poly I:C)

The initial clinical trial with this interferon inducer has now been completed. Thirty patients were studied at 11 dose steps, ranging from 0.3 mg/m^2 to 75 mg/m^2, intravenously. Additional studies were performed by giving the drug by the intramuscular route and by inhalation. The following have been noted:

a. Interferon induction is not linear with increasing doses beyond 8 mg/m^2.

b. After an initial dose, a refractory period occurs with further drug administration during which there is no detectable interferon produced.

c. To prevent this refractory period, second doses need to be spaced out longer than 1 week.

d. Interferon was not detectably induced when doses equivalent to effective intravenous doses were given intramuscularly.

e. At doses of 12 mg/m^2 interferon was not detected after inhalation of Poly I:C.

f. The initial concern about the toxicity secondary to intravascular coagulation did not prove dose limiting up to the top dose, 75 mg/m^2. Toxicity was as follows:

1). Progressive, reversible anemia

2). Fever, headaches, nausea and vomiting

3). Occasional reversible abnormal liver functions

4). Clotting abnormalities were noted in the test tube, but were not clinical problems.

The drug was tested in tumors known to be resistant to most current agents, and no therapeutic benefit has been noted.

5. The Nitrosoureas

The study of BCNU is reported in the section on Hodgkin's disease. The cyclohexyl nitrosourea phase I study was completed this year in cooperation with the Veterans NCI ONcology Unit and is reported in their project report.

A new nitrosourea derivative, methyl cyclohexyl nitrosourea, is available and about to undergo clinical trial. This compound is of interest because it is the only nitrosourea derivative with a spectrum of activity different from BCNU and CCNU. Thus, in animal tumor systems, this drug is effective against the very drug resistant Lewis lung carcinoma. The phase I study has just been initiated.

6. Procarbazine

Procarbazine is a methyl hydrazine derivative effective in the treatment of Hodgkin's disease and in some cases of oat cell carcinoma of the lung. Heretofore, it has only been available for use by the oral route. Since current evidence suggests that large pulses of active drugs are superior to the same agent given by the daily oral schedule, we have instituted a phase I trial with the newly available parenteral preparation of procarbazine. Patients have been placed at single, weekly doses ranging from 300 to 1000 mg/m^2 with no dose-limiting toxicity to date. This study will continue into the next year.

C. Clinical Investigation, Miscellaneous

1. Staging in Lymphomas

a. Hodgkin's Disease

Diagnosis of disease outside of the lymph node areas has been a problem in the past, and at many institutions investigators have turned to the use of exploratory laparotomy to identify disease in the liver. In the past year we embarked on two studies to examine the ability to diagnose disease in the liver of patients with Hodgkin's disease.

1). The first approach was to examine all past liver biopsies in patients with Hodgkin's disease and correlate these findings with prebiopsy assessment of the liver and ultimately proven organ involvement.

This study confirmed the impression that liver function studies are valueless in predicting tumorous involvement of the liver.

A finding of interest is that the presence of atypical reticulum cells in the absence of Reed-Sternberg cells was an accurate predictor of early subsequent liver involvement, and this finding may serve as diagnostic evidence of the presence of Hodgkin's disease in the liver.

2). Peritoneoscopy was instituted last year to avoid laparotomy in the staging of Hodgkin's disease. The results have been useful, and it appears that for purposes of diagnosing liver involvement, peritoneoscopy is as good as, or perhaps superior to, laparotomy. Only 4 percent of percutaneous biopsies were positive in the past. With peritoneoscopy, 20 percent of 40 patients with Hodgkin's disease undergoing peritoneosocpy have positive biopsies, an incidence slightly higher than found at laparotomy. We have been able to identify a high-risk group of patients for liver involvement; those with some evidence of disease below the diaphragm (positive lymphangiograms and/or a palpable spleen), prior to the procedure.

b. Lymphosarcoma and Reticulum Cell Sarcoma

Because of the rarity of truly localized disease, we have embarked on an intensive staging workup in these diseases. The aim is to test the relative efficacy of routine workup versus the addition of peritoneoscopy, and, ultimately in patients with negative peritoneoscopy, laparotomy. To date it appears that laparotomy added to a negative peritoneoscopy does not identify additional disease.

2. Reproductive Capacity of Patients with Malignancies Successfully Treated for their Disease

With the increase in the success rate of treatment of advanced Hodgkin's disease and other tumors, we now have a significant number of young individuals alive and free of disease at 5 years. Many of these patients inquire about their reproductive capacity, and since there is little data on which to base an answer, we have taken the opportunity to study patients in remission after chemotherapy to gain insight into the problem, and also ask some significant questions about physiologic feedback mechanisms in the control of spermatogenesis. Of additional significance in these studies is that spermatogenesis and sperm production represents a model of a stem cell pool very similar to bone marrow.

We have studied 12 such men with lymphomas in remission for 1-5 years after treatment to evaluate the interaction of the testicular components and gonadotropin secretion. Persistent azoospermia was present in 80 percent of the men. Testicular biopsy in these azoospermic men showed complete absence of all germinal elements; however, Sertoli cells and Leydig cells were present. Plasma FSH was elevated 5 fold in each man, compared to normal men. One patient with a normal sperm count 2 years after chemo-

therapy had complete spermatogenesis on biopsy and normal plasma FSH.

In contrast, serum luteinizing hormone was normal in all men, and plasma testosterone was normal in all but one. Furthermore, clomiphene, 200 mg daily for 5 days, produced increases in serum FSH, LH, and plasma testosterone, demonstrating appropriate pituitary-Leydig cell responses.

An additional azoospermic patient with lymphoma had received a full course of chemotherapy 2 months before study, but was not in remission. Plasma FSH was normal, despite the presence of only spermatogonia and Sertoli cells in the testicular biopsy, suggesting that the latter stages of spermatogenesis do not affect FSH secretion. As with the other men, serum luteinizing hormone, testosterone, and responses to clomiphene were normal.

Since FSH levels are lower in these patients than in castrate men, we conclude that, although FSH may be modulated in part by Leydig cells and/or testosterone, other factors related specifically to the germinal epithelium are important. We suggest that the source of a testicular factor involved in FSH feedback control is either the Sertoli cell or the early germinal elements.

Honors and Awards:

Dr. Charles Bagley

1. "Combination Chemotherapy with Cyclophosphamide, Vincristine, and Prednisone in Lymphosarcoma." Presented at the 62nd Annual Meeting of the American Association for Cancer Research, Chicago, Ill., April 1971.

Dr. Vincent T. DeVita

1. "Combination Chemotherapy of Advanced Hodgkin's Disease: Effect on Survival." Presented at the 13th International Congress of Hematology, Munich, Germany, August 1970.

2. "Hodgkin's Disease and Other Lymphomas: Natural History of the Disease and Chemotherapy." Presented at the Annual Oncology Symposium. Nassau Hospital, Mineola, New York, October 1970.

3. "Combination Chemotherapy of Hodgkin's Disease." Presented at the Annual Meeting of the American Society of Therapeutic Radiologists, Phoenix, Arizona, November 1970.

4. "A New Look at the Chemotherapy of the Lymphomas." Presented at the Annual Cancer Seminar, Doctors Hospital, Washington, D. C., January 1971.

5. "Evaluation of the Diagnosis, Staging, and Therapy of Hodgkin's Disease in the Past Decade." Presented at the Annual Meeting of the St. George Society, Washington, D. C., March 1971.

6. "The Management of Malignant Lymphomas: Chemotherapy." Presented at the Annual Cancer Seminar of Springfield Hospital, Springfield, Mass., March 1971.

7. "Perspectives in the Treatment of Carcinoma of the Ovary." Presented at the VI Annual Cancer Conference of the Akron City Hospital, March 1971.

8. "The Management of Hodgkin's Disease." Presented at the VI Annual Cancer Conference of the Akron City Hospital, Akron, Ohio, March 1971.

9. "Newer Approaches to the Treatment of Hodgkin's Disease." Presented at the Annual Meeting of the American College of Physicians, April 1971.

10. "Therapeutic Implications of Staging: Chemotherapy." Presented at the Conference on the Staging of Hodgkin's Disease, Ann Arbor, Michigan, April 1971.

11. "Peritoneoscopy in the Staging of Hodgkin's Disease." Presented at the Conference on the Staging of Hodgkin's Disease, Ann Arbor, Michigan, April 1971.

12. "The Role of Chemotherapy in the Management of Patients with Lymphomas." Presented at the Seminar on Lymphomas, Pacific Northwest Research Foundation, Seattle, Washington, May 1971.

13. "Chemotherapy of Hodgkin's Disease and Other Lymphomas." Presented at Mt. Sinai School of Medicine Course in Modern Clinical Cancer Chemotherapy, New York, May 1971.

Dr. David Van Thiel

1. "Evidence for a Specific Seminiferous Tubular Factor Affecting FSH Secretion in Man." Presented at the Annual Meeting of the Endocrine Society, San Francisco, California, June 1971.

Dr. Robert C. Young

1. "The Treatment of Carcinoma of the Ovary." Presented at Fairfax Hospital, March 1971.

2. "The Treatment of Advanced Hodgkin's Disease with BCNU 1,3 Bis (2Chloroethyl)-1-Nitrosourea." Presented at the Annual Meeting of the American Society of Clinical Oncology, Chicago, Illinois, April 1971.

Dr. George P. Canellos

1. "The Chemotherapy of Cancer: Practical Applications." Presented at the VI Annual Cancer Symposium, Akron City Hospital, Akron, Ohio, March 1971.

2. "The Burkitt Lymphoma." Presented at the VI Annual Cancer Symposium, Akron City Hospital, Akron, Ohio, March 1971.

3. "Hypercalcemia in Patients with Cancer." Presented at the VI Annual Cancer Symposium, Akron City Hospital, Akron, Ohio, March 1971.

Publications:

1. Hill, D. A., Baron, S., Levy, H. B., Bellante, J., Buckler, C. E., Canellos, G. P., Carbone, P., Chanock, R. M., DeVita, V. T., Guggenheim, M., Homan, E., Kapikian, A., Kirchstem, R. L., Mills, J., Perkins, J. C., Van Kirk, J., and Worthington, M.: Clinical studies of induction interferon by polyinosinic-polycytidylic acid. Proc. Curtav Stern Symposium of Current Aspects in Virology. February, New York, 1970.

2. Vogel, C. L., DeVita, V. T., Denham, C., Foley, H. T., Field, R. B., and Carbone, P. P.: Preliminary clinical trials and clinical pharmacology studies with oral imidazole 4(or 5) carboxamide 5(or 4), 3 Bis (2-chloroethyl)1-triazeno NSC 82196. Cancer Chemother. Rep. (in press).

3. DeVita, V. T., Serpick, A. A., and Carbone, P. P.: Combination chemotherapy in the treatment of advanced Hodgkin's disease. Annals of Int. Med. 73:881-895, 1970.

4. DeVita, V. T.: Therapeutic implications of staging: Chemotherapy. Cancer Res. (In press).

5. DeVita, V. T., Bagley, C. M., O'Kieffe, D. A., and Trujillo, N. P.: Peritoneoscopy in the staging of Hodgkin's disease. Cancer Res. (In press).

6. Carbone, P. P., and DeVita, V. T.: Malignant lymphoma. Cancer Medicine (In press).

7. Lowenbraun, S., DeVita, V. T., and Serpick, A. A.: Combination chemotherapy with nitrogen mustard, procarbazine, and prednisone in previously treated patients with Hodgkin's disease. Blood 36:704-717, 1970.

8. DeVita, V. T., and Goodell, B. W.: Chemotherapy of Hodgkin's disease. In Conn, H. F. (ed.) Current Therapy. Philadelphia, W. B. Saunders Co., 1971, pp. 234-239.

9. Bagley, C. M., Roth. J. A., Thomas, L. B., and DeVita, V. T.: Clinico-pathologic correlations of liver biopsies in Hodgkin's disease. Clinical Res. 19:489, 1971

Serial No. NCI-3404
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Immunologic Aspects of Cancer

Previous Serial Number: Same

Principle Investigators: Vincent T. DeVita, Robert C. Young, Michael Corder, John E. Bennett

Cooperating Units: Clinical Investigations Branch, NIAID

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

A. Infections and Cancer

To identify, define, and control infectious complications associated with the use of immunosuppressive agents in the treatment of patients with cancer.

B. Immunology and Cancer

To study the immunologic status of patients with malignancies, and relate these findings to progression or arrest of disease and survival. To exploit the hosts immunologic system as an adjuvant to the chemotherapy of tumors.

C. Infections

1. Pneumocystic Carinii

The results of four years of investigations into the identification and treatment of this illness were described in last years Annual Report. Currently, the case accrual continues to be high and our series represents the largest single experience with this illness. The results of therapy, side effects and long term outlook of patients with this form of pneumonia are being collated as our final contribution to its management. To date, no patient has developed a second episode of infection with this organism

suggesting that a permanent immunity may develop and raise the question of immunization of high risk cancer patients prior to therapy.

2. Aspergillus Fumigatus

Continuing interest in the problem of Aspergillosis in patients with malignant diseases has taken the form of a search for evidence of antibody response in patients with invasive Aspergillosis. Serological tests of this nature would be extremely helpful in the early diagnosis of this commonly fatal complication of malignant disease. A search for an antibody response to invasive Aspergillosis was made in 15 patients with wide-spread invasive Aspergillosis using techniques of double difusion in agargel, compliment fixation, immunoelectrophoresis and indirect fluorescent antibody. Parallel control studies using similar tests, demonstrated specific anti-aspergillus antibody of the IgG type in serum from a patient with non-invasive bronchopulmonary aspergillosis. In contrast, no antibodies to aspergillus fumigatus were detected in the sera of these 15 patients with widespread invasive aspergillosis. Current enthusiasm for the serologic diagnosis of aspergillosis has derived largely from studies in clinical forms of the disease in which the fungus shows little tendency to penetrate living tissue. In patients with invasive aspergillosis, however, there appears to be no evidence of an antibody response to the infection by the usual serological methods now available.

Two other aspects of the problem of aspergillosis infections are currently being studied. The first is an attempt to assess the significance of fungus cultures in our patient population, the second is an analysis of the characteristics of the clinical disease caused by the various species of aspergillus.

B. Immunology and Cancer

1. The Immunology of Hodgkin's Disease

Delayed hypersensitivity in Hodgkin's disease was examined in 103 untreated patients in all stages of the disease. Cutaneous anergy was uncommon in these patients, only 11.7% had no reaction to any of the six skin tests supplied. No stage I patient was anergic; the incidence of anergy increased with stage, but only 26.6% of the stage IV patients were anergic. Mumps and DNCB were the most likely skin tests to rule out anergy. Skin test reactivity correlated with the absence of systemic symptoms as well as with histological type. Patients with nodular sclerosis and lymphocyte predominant patterns have a higher incidence of skin tests reactivity than do patients with mixed cellularity and lymphocyte depleted Hodgkin's disease. Absolute peripheral lymphocyte counts were noted to reflect staging and skin test reactivity. The mean absolute lymphocyte counts fell with progressive stage and skin test reactivity generally increased with increasing peripheral lymphocyte count. For the first time, it was

possible to analyze the influence of the initial skin test reactivity on prognosis and survival in patients with Hodgkin's disease. In summary, skin test reactivity at the time of initiation of therapy was not found to influence either prognosis or survival. Peripheral lymphocytopenia at the onset of the patients course, however, did seem to be a bad prognostic sign.

In a second manuscript, we are currently summarizing our experience with PHA-induced lymphocyte stimulation in patients with Hodgkin's disease and correlating their initial reactivity to prognosis and survival. It would appear that PHA stimulation is most markedly impaired in stage IV patients with Hodgkin's but the initial ability to respond to PHA stimulation within a given stage of the disease does not correlate well with prognosis and survival.

2. The Etiology of the Anergic State

Previous studies in our laboratory have revealed a close correlation between the presence of the anergic state and abnormalities of pyridoxine balance in patients with Hodgkin's disease. Clinical studies by Axelrod have indicated that induced pyridoxine deficiency can lead to failure to elicit a delayed hypersensitivity response and to lymphocytopenia. It is possible that progressive anergy occurs as a result of the progression of a malignancy, rather that the reverse, and may be related to a metabolic defect. Our studies with skin testing in Hodgkin's disease, previously cited, suggest that this may be true.

A pyridoxine deficient diet has been obtained and certain patients with known normal skin test reactivity will be made pyridoxine deficient to test the ability to induce anergy and restore the defect with reconstitution of pyridoxine in the diet.

Honors and Awards:

Dr. Vincent DeVita

1. "Pulmonary Infections in Immunosuppressed Patients." Presented at Lung Seminars. George Washington University Medical School, January 1971.

Publications:

1. DeVita, V.T., Chabner, B.A., Livingston, D.M., and Oliverio, V.T.: Anergy and tryptophan metabolism in Hodgkin's disease. Amer. J. of Clinical Nutrition (In press).

Serial No. NCI-3405
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chronic Myelogenous Leukemia

Previous Serial Number: Same

Principle Investigators: George P. Canellos, Vincent T. DeVita, Jacqueline Whang

Cooperating Units: Surgery Branch, NCI
Human Tumor Cell Biology Branch, NCI

Man Years:

Total:	2.5
Professional:	2.0
Other:	0.5

Project Description:

This disease has been the subject of investigation in this unit for some years. This is a neoplasm which is of great interest to a medical oncology unit because:

(1) it is a disease whose cells can be studied chronically;
(2) its evolution to a more malignant phase mirrors the natural history of many other human tumors. The evolution from hyperplasia to true neoplasia is an ideal model for biochemical studies of the mechanisms of differentiation.
(3) chromosomal studies can be conducted serially and,
(4) leukocytes can be harvested for preservation studies and subsequent transfusion.

Although the chronic phase can be controlled by chemotherapy, the blastic transformation has been an extremely difficult phase of the disease to control and represents a challenge similar to acute myelogenous leukemia. Increasing efforts in tissue typing and blood cell preservation offer a unique opportunity in this disease, since it is one where the likelihood of developing acute leukemia within 5 years of diagnosis is at least 50-60 percent. It is attractive to postulate that hyperplastic marrow could be banked and reutilized at the time of blastic crisis if chemotherapy failed to remit the disease. Such a program is in progress.

A. Correlations of Cytogenetics and Natural History

The specific Philadelphia (Ph') chromosome marker in CML is well known. Serial studies of CML through the natural evolution to blastic transformation were performed on 60 patients. Sixty-four percent of the patients developed evidence of new, but closely related karyotypes still bearing the Ph' chromosome, suggesting clonal evolution of blastic cell lines. In addition, evidence for clonal evolution was obtained by the detection of aneuploid Ph' blast cell lines in extramedullary foci of blastic crisis prior to systemic involvement. Serial cytogenetic studies during blastic transformation revealed evidence of increasing genetic variation with a succession of aneuploid lines.

B. Therapeutic Progress

1. Chronic Phase

We have a comparative study of Myleran and Dibromomannitol, a new effective agent in CML. Thirty-three patients have been entered and are currently being followed. The incidence of complete remission between the two groups is comparable. There does not appear to be a difference in the temporal occurrence of blastic crisis in the groups. Patients sensitive to the thrombopenic effects of Myleran are similarly sensitive to DBM. Recovery from leukopenia appears to be more rapid from DBM than Myleran. Long-term dysplastic effects of Myleran were recorded.

2. Splenectomy During the Chronic Phase

Splenomegaly, splenic infarction, splenic sequestration of RBC and platelets, and abdominal pain characterize the accelerated phase of CML. Similarly, effective chemotherapy during the blastic phase is limited by thrombopenia, anemia, and the increased tumor cell mass because of massive splenomegaly. Previous experience in six cases with thrombopenia during the chronic phase indicated that the platelet count could be improved by splenectomy. Thirteen patients have undergone prophylactic splenectomy without difficulty. Granulocyte sequestration with Cr_{51} labelled autologous cells were performed in four patients. This study will continue in future years to evalute the effect of prior splenectomy on the ability to treat the blastic phase of CML. There has been no mortality or morbidity from the operation. Three patients have died in blastic crisis. Two failed to respond to chemotherapy, yet survived 5, 7 months even without remission.

3. Chemotherapy of the Blastic Phase of CML

The previous experience in the chemotherapy of the blastic phase of CML has been limited by the prolonged leukopenia and thrombopenia and

myelofibrosis in some cases. An attempt to increase remission with agents without marked myeloid or platelet toxic effects was undertaken. Vincristine and prednisone were administered to 30 consecutive patients. Six patients achieved hematologic and cytogenetic remission of aneuploid cell lines, a previously rare event in the management of blastic crisis. Three other patients achieved a good partial remission. There appeared to be a correlation with early diagnosis, absence of significant splenomegaly, and cytogenetic evidence of two cell populations. The survival of responding patients is significantly longer statistically than previously experienced with POMP, BCNU/ARA-C, with a median survival of 10+ months.

Since there appeared to be a correlation between response to vincristine and prednisone, and hypodiploidy, it was decided to randomize a new group of patients between vincristine/prednisone and Cytosine arabinoside/thioguanine in order to test whether another regimen found to be effective in AML may be effective against either the same type of patient who responds to vincristine and prednisone. This study was instituted this year.

C. Biochemical Studies of CML Leukocytes

1. Abnormality of B_{12}-binding in CML: A protein marker of myelopoiesis

Studies in the past have revealed that CML patients have high serum B_{12} levels. Secondly, this appears to be due to an increase in an alphaglobulin B_{12}-binding protein (Transcobalamin). In vitro studies have, therefore, demonstrated C_{12}-amino acids are incorporated into leukocyte proteins with chromatographic characteristics of the serum B_{12}-binding protein. In addition, the addition of CML leukocytes to normal serum alters the B_{12}-binding capacity to that of leukemic serum. Studies are in progress to demonstrate this protein to be leukocyte membrane origin and to be a glycoprotein.

The interesting extension of this work is to develop a marker of myeloid maturation for in vitro studies. The physiologic importance of transcobalamin I is yet undefined. It is possible that the convenient binding of B_{12} is nonphysiologic and demonstrates that leukocytes can elaborate proteins which may have physiologic or kinetic importance -- perhaps a leukocyte kinin or chalone.

The availability of large numbers of platelets from CML patients who have had previous splenectomy permitted a study of the B_{12}-binding proteins of such platelets. A protein binding with elution characteristics identical with the leukocyte protein was found. A collaborative investigation (Dr. Paul Nieman, University of Washington, Seattle), related to the B_{12}-binding problem was the conclusive demonstration that transcobalamin II, a Beta globulin, is the transport protein which is

physiologically active. We are reporting a study in two siblings, both of whom lack this protein with resulting severe marrow hypoplasia correctable only with massive amounts of B_{12} given weekly.

2. Stem Cell Function in CML

Chronic phase of CML appears to be a disorder of stem cell proliferation since maturation appears to be normal. The growth of CML in soft agar or methylcellulose has been demonstrated and continues. The effect of erythroprotein on marrow and peripheral blood erythroid cells was undertaken. Marrow erythroid cells do not respond, whereas peripheral blood erythroid precursors do stimulate. A similar nonstimulatory pattern was seen in polycythemia vera marrow. Investigations continue on the mechanism.

3. Cell Kinetic Studies

Cell kinetic studies are in progress in patients with the acute phase of CML to obtain information on the life cycle of the stem cell line. These studies are described in project 6075.

4. Collaborative Studies

a. Leukocytes have been provided for a variety of investigators interested in leukocyte biochemistry.

b. Delayed hypersensitivity studies to CML leukocytes were investigated by Immunology Branch investigators.

Honors and Awards:

Dr. George P. Canellos

1. "Chronic Granulocytic Leukemia." Presented at American Society of Hematology Education Session. Puerto Rico, December 1970.

2. "Chronic Granulocytic Leukemia: Clinical and Cytogenetic Correlations." Presented at XIII International Society of Hematology. Munich, Germany, August 1970.

3. "Cytogenetics and Hematologic Remission of Blastic Transformation in Chronic Granulocytic Leukemia." Presented at the American Society of Clinical Oncology. Chicago, Ill., April 1971.

4. "Treatment of Blastic Transformation of CGL." Presented at Georgetown University. March 1971.

5. "Cytogenetic Remission of Blastic Transformation." Presented at Tenth International Cancer Congress. Houston, Texas, May 1970.

Publications:

1. Henderson, E.A., and Canellos, G.P.: Diagnosis of leukemia: Chronic leukemias. Conn, H. (Ed.): Current Diagnosis. 1970.

2. Haskell, C.M., Canellos, G.P., and DeVita, V.T.: Hypercalcemia in leukemia. Cancer (In press).

3. Carbone, P.P., and Canellos, G.P.: Therapy of chronic granulocytic leukemia. Revue de Medicine 11: 295-298, 1970.

4. Canellos, G.P., and Cohen, M.P.: A vitamin B_{12}-binding protein derived from platelets of chronic graunulocytic leukemia. Clinical Res. 19: 413, 1971.

Serial No. NCI-3420
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Kinetics of Cellular Proliferation

Previous Serial Number: 6075

Principle Investigator: Vincent T. DeVita

Other Investigators: Robert C. Young, Charles Bagley, Richard Adamson, Vincent Oliverio, and George Canellos

Cooperating Units: Acute Leukemia Service, NCI
Laboratory of Chemical Pharmacology, NCI

Man Years:

Total:	2
Professional:	1.5
Other:	0.5

Project Description:

Information derived from animal tumor systems has clearly shown that interrelating knowledge of the biology of tumor growth (cell kinetics) with therapy improves therapeutic responses. Much of the application of this knowledge to humans has been hampered by the lack of human data.

The goals of the tumor cell kinetic program of the Solid Tumor Service have been as follows:

1. To examine and develop methodology that might be useful in obtaining cell kinetics information in individual patients in a time period that may allow application of these data to the patient's therapy.

2. To describe the kinetics of human tumors, compare them to animal models, and to compare results to normal tissues, to the same tumor in different patients, and to different metastatic sites of the same tumor in the same patient.

3. To examine the manner in which cell cycle characteristics are changed in response to exposure to drugs.

4. To approach the problem of manipulating tumor growth characteristics in such a way as to increase its susceptibility to chemotherapeutic agents.

In the past two years we have been able to:

1. Develop methodology for rapidly defining tumor cell kinetics in human tumors.

2. Collect information, using these methods, in carcinoma of the breast, malignant melanoma, and the acute and chronic leukemias.

3. Show that cell cycle characteristics can be altered by chemotherapeutic agents, particularly the nitrosoureas.

This year's efforts have been directed to further collection of data in human solid tumors, and developing the Lewis lung tumor model for studies in perturbation of cell cycles.

Results:

A. Animal Studies

1. Cell cycle perturbation

Two tumors have been used to study the effects of hyperbaric oxygen on the growth fraction and duration of cell cycles; these are the Adamson reticulum cell sarcoma and the Lewis lung carcinoma. To date, hyperbaric oxygen has not successfully perturbed the growth characteristics of these tumors. Other methods of cycle perturbation will be investigated in the coming year.

2. Alteration in growth characteristics of leukemia L1210 by the nitrosoureas.

We have previously shown that the duration of S phase is prolonged in L1210 leukemia by exposure to the nitrosoureas. Regrowing tumors restored their cell cycle to normal, but regrowth occurred with a lower proliferative capacity, as manifested by a reduction of the growth fraction from 100 to about 30 percent. The evidence that an actual persistent change in the cell cycle itself was unclear. We also demonstrated that the effect on the cell cycle was produced by the isocyanate portion of the nitrosourea molecule.

Studies have been performed in the past year that show that the cell cycle of regrowing nitrosourea exposed cells which is subsequently transplanted and allowed to regrow are prolonged. The data suggests that either a real persistent prolongation of the cell is produced or the chemotherapeutic agent leads to the selection and survival of a tumor line with a longer cell cycle.

3. Modification of normal marrow kinetics by hormonal agents

a. Effect of androgens on granulopoiesis

The ability of the normal marrow to tolerate toxic antitumor agents is a continuous limitation in the application of adequate doses of active agents to sensitive tumors. There is a pressing need to have the ability to modify the cellular growth pattern in normal and malignant cells in order to facilitate the use of chemotherapeutic agents. To that end an extensive study of the effect of corticoids and androgens on the kinetics, colony forming ability, and relative vulnerability to cell cycle active agents is being undertaken in the marrow cells of normal mice treated in vivo with these agents.

We have already demonstrated an increased cloning ability of marrow derived from androgen-treated animals. Since this assay may well select for cells active in cycle or in DNA synthesis, we are determining whether androgens may, in fact, increase vulnerability to cytotoxic drugs or conversely by increasing a "stem" cell pool can increase the tolerance.

b. Humoral control of granulopoiesis: Cell cycle specificity

In order to extend our knowledge in the control of granulopoiesis and marrow function, a project to test the humoral-control agents, such as erythropoietin and a granulopoiesis-promoting factor are more active during certain phases of the cell cycle in the mouse. A mouse mammary tumor (MACA1460) has been demonstrated to produce granulopoiesis by a "humoral" factor. The tumor is now carried in CE mice and is being harvested and frozen. The plasma of the mice is also pooled and frozen. The marrows of these animals have been shown to have a high colony forming ability in vitro. This factor is being prepared and purified for use in vitro culture system.

4. Summary of Current Approaches to Animal Cell Kinetic Studies

Since the methodology for animal tumor studies is far more convenient than human tumors, we will continue, in the next year, to examine methods of perturbing cell cycles by oxygen, nutrients, hormones, and growth promoters, and combine these studies with comparative cell kinetics data on normal target tissue, such as marrow and gastrointestinal tract. Methods of protecting marrow with simultaneous stimulation of tumors will continue to be sought.

B. Studies in Man

1. Acute leukemia and the blastic phase of chronic myelogenous leukemia

The use of the in vivo double labelling technique with direct injection of isotope into the bone marrow of patients with leukemic involvement of the marrow has allowed the collection of rapid cell kinetic information in an individual patient. Over 15 patients have been studied with his approach. In the past year it has been possible to perform growth fraction studies by inserting a plastic catheter directly into the bone marrow cavity for repeating pulsing, prior to a single sampling to determine growth fraction.

Such studies can now be advanced for the stage where cell kinetic parameters can be related to response to treatment, survival, type of drugs used, etc., with the rapid relation of the data to the results in an individual patient.

2. Solid Tumors

The descriptive phase of cell kinetics in human solid tumors has continued with the examination of the cell cycles of additional patients with breast cancer, melanoma, and reticulum cell sarcoma. These studies have incorporated repeated pulsing for growth fraction's determination in addition to labelled mitoses curves.

All the data in solid tumors are consistently showing the following:

a. S phases are fairly comparable 19 to 25 hours in all tumors.

b. Significant variability around the mean cycle length occurs expressed as marked dumping of the PLM curves.

c. Second waves of labelled mitoses are not noted; this probably is an expression of the marked variation in cycle times.

d. Growth fractions, at least in skin lesions, are surprisingly high and may account for the general propensity of skin lesions to respond to treatment more often than visceral lesions.

3. Summary of Human Data

We appear to be at a cross-roads in the study of human tumor cell kinetics

a. Descriptive studies are revealing a unanimity of results confirming the differences of human and animal tumor cell cycles.

b. Further studies in this area seem unlikely to directly aid therapeutic programs.

c. Studies employing kinetic methods to measure perturbation of growth characteristics and recruitment of cells into cycles would appear to offer the most opportunity for progress.

d. The key to future knowledge of tumor cell growth appears to revolve around the measurement of the numbers of surviving clonogenic cells in a tumor.

Honors and Awards:

Dr. Vincent T. DeVita

1. "Cell Kinetics and Cancer." Presented at the First Joint Working Conference, Annapolis, Md., December 1970.

2. "Basic Principles of Chemotherapy: Cell Kinetics." Presented at the 6th Annual Cancer Symposium of the Akron City Hospital, Akron, Ohio. March 1971.

3. "Cell Kinetics and Chemotherapy." Presented at the Joint Meeting of the VA Lung Cancer Group and VA Surgical Adjuvant Group. April 1971.

Publications:

1. Bray, D.A., DeVita, V.T., Adamson, R., and Oliverio, V.T.: Effects of 1, (2 chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and its degradation products on progression of L1210 cells through the cell cycle. Cancer Chemother. Rep. (In press).

2. DeVita, V.T.: Cell kinetics and the chemotherapy of cancer. Cancer Chemother. Rep. (In press).

Serial No. NCI-3421
1. Medicine Branch
2. Solid Tumor Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Study of the Physiologic Disposition, Metabolism, Excretion, and Metabolic Interaction of Antitumor Agents

Previous Serial Number: 6076

Principle Investigators: Vincent T. DeVita, George P. Canellos

Other Investigators: Sheldon Wolfe

Cooperating Units: NIAID

Man Years:

Total:	1.5
Professional:	1.0
Other:	0.5

Project Description:

A. L-asparaginase

The phase I evaluation of this compound is completed. In addition to the clinical studies an extensive pharmacologic and biochemical study was performed in order to study:

1. The disposition of the drug after a large single dose and its effect of circulating plasma asparagine and red cell asparagine. To explore any differences amongst patients in the clearance of the agent and the rate of plasma asparagine recovery.

2. Mechanism of biochemical resistance via the enzyme asparagine synthetase. Mechanisms of induction, effect of inhibition, kinetics of the enzyme and its induction.

3. The lack of myelotoxicity was studied in mice by means of marrow cultures and enzyme analysis. A dose of 1,500 I.U. per kilogram has an apparent volume of distribution of 4.8-7.0 liters with plasma half time of 1.1-1.4 days. Trace amounts remained in the plasma up to 3 weeks after the single dose. Plasma asparagine was undetectable for three weeks in 3/4 patients. The amino acid was studied serially in leukemic cells of 4 patients and was undetectable during treatment. Repeat pharmacologic studies were performed on a crystalline preparation of the enzyme.

Asparagine synthetase was studied in 19 leukemic patients and was found to be low or absent prior to therapy. Following treatment a 6-fold rise in the enzyme occurred in resistant cells and remained unchanged in the sensitive cells. A human enzyme, asparagine synthetase was partially purified from KB cells in culture. Co-factor requirements and the effect of asparagine antagonists were similar to other mammalian sources. The glutamine antagonists, DON and azotomycin were found to be effective inhibitors of the enzyme while azaserine was relatively inactive.

The tolerance of normal marrow cells to asparagine was studied in normal mice. The ability of myeloid stem cells to tolerate antileukemic effects of the drug was documented by an in vitro colony assay technique. The drug was found to bind closely to cell membranes and normal marrow cells can be rapidly "turn on" asparagine synthetase activity.

B. Procarbazine

The ability of this hydrazine derivative to oxidize sulfhydryl groups was studied in intact erythrocytes. In order to help define its potential clinical toxicity when used in patients with erythrocyte glucose-6-phosphate dehydrogenase deficiency and to utilize the human RBC as a model for the effect of tumor drugs on membranes. Incubation with procarbazine caused rapid oxidation of glutathione in the RBC and the formation of methemoglobin with increased osmotic fragility. Membrane -SH groups were unaffected by oxidizing levels of drug. G-6-Pd cells were more sensitive than normal. Glucose protects the cell from these effects. In the presence of oxyhemoglobin the drug, generates H_2O_2 which appears to be the mechanism for RBC toxicity.

C. Imidazole mustard (NSC 82196)

The radiopotentiating effect of this drug was studied in L1210 leukemia utilizing a variety dose schedule. Utilizing 100 g/kgm I.P. for 5 days followed on day #5 with 300r TBR resulted in 20% cures with none of the controls surviving. The drug and radiation given together showed no benefit.

In conjunction with the initial clinical trials of this drug given by the oral route pharmacologic studies were conducted with radiolabelled compound (14C). These studies indicated extremely erratic absorption by the oral route which was consistent with the erratic toxicity observed. The oral preparation was abandoned and clinical trials are in progress with the parenteral form. (See clinical trials section)

D. Cyclophosphamide

Studies with 14C cyclophosphamide are continuing. Past studies have concentrated on looking for differences in ability of patients to

metabolize the compound as an explanation of differential response rates. None were found. Current studies are in progress to study the pharmacokinetics of the drug given by different routes and schedules. In cooperation with Dr. Sheldon Wolfe of NIAID, a group of patients with Wegeners granulomotosis who have received cyclophosphamide for up to five years are being studied to determine the effect of long term administration on the ability to metabolize the compound. These patients have been observed to be less tolerant to the drug with increasing time; four such patients have been studied thus far.

Further studies with induction and inhibition of the metabolism of this compound are in progress.

Publications:

1. Haskell, C.M., and Canellos, G.P.: Asparagine biosynthesis in human KB tumor cells: Inhibitor studies with asparagine and glutamine antagonists. Cancer Res. 30: 1081, 1970.

2. Hansen, H.H., and Canellos, G.P.: L-asparaginase treatment of acute myeloblastic leukemia. Oncology 5: 349, 1970.

3. Haskell, C.M., Canellos, G.P., Cooney, D.A., and Hansen, H.: Biochemical and pharmacologic effects of L-asparaginase in man. J. Lab. Clin. Med. 75: 763, 1970.

4. Brown, C.L., Canellos, G.P., and Carbone, P.P.: Effect of L-asparaginase in mouse bone marrow assayed by in vitro culture. Blood 36: 385, 1970.

5. Canellos, G.P., and Haskell, C.M.: Studies of resistance to L-asparaginase in human leukemia. Recent Results in Cancer Research (Springer-Verlag) 33: 188, 1970.

6. Vogel, C.L., DeVita, V.T., Denham, C., Foley, H.T., Field, R.B., and Carbone, P.P.: Preliminary clinical trials and clinical pharmacology studies with oral imidazole 4 (or 5) carboxamide 5 (or 4), 3 Bis (2-chloroethyl)- 1-triazeno NSC 82196. Cancer Chemother. Rep. (In press).

7. DeVita, V.T., and Adamson, R.H.: The metabolism of 14C cyclophosphamide comparative drug metabolism studies on African and American patients with lymphsarcoma and Burkitt's tumor. Progress in Antimicrobial and Anticancer Chemotherapy. Tokyo, Japan. University of Tokyo press 1970, Vol II, pp. 218-225.

8. DeVita, V.T.: BCNU (1,3-bis (2 chloroethyl)-1-Nitrosourea: Clinical studies and pharmacology. In Carter, S.K. (Ed.) Proceedings of the Chemotherapy Conference on the Chemotherapy of Solid Tumors; an

Appraisal of 5-Fluorouracil and BCNU, pp. 181-193, 1970.

9. Canellos, G.P., Sponzo, R.W., and Arseneau, J.: Oxidative hemolytic effect of procarbazine in vitro. Clinical Res. 19: 489, 1971.

Serial No. NCI-3422
1. Medicine Branch
2. Uganda Cancer Institute
3. Kampala, Uganda

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Study of Burkitt's Tumor, Hodgkin's Disease, and Malignant Melanoma

Previous Serial Number: Same

Principal Investigators: John L. Ziegler, Avrum Bluming, and Charles L. Vogel

Other Investigators: Sebastian Kyalwazi, Paul P. Carbone, Ian McAdam, and Charles L. Olweny

Cooperating Units: Pathologic Anatomy Branch, NCI; Immunology Branch, NCI; Laboratory of Chemical Pharmacology, NCI; Departments of Surgery, Pathology and Medicine, Makerere University, Kampala, Uganda

Man Years:

Total:	9
Professional:	3
Other:	6

Project Description:

Objectives:

The major purpose of this research project is to obtain quantitative information regarding the therapy of patients with Burkitt's tumor, and other neoplastic diseases in Uganda that would be of direct interest to the treatment of cancer in the United States. The Lymphoma Treatment Center was set up in August, 1967, under contract PH43-67-47 and expanded to include the Solid Tumor Center in December, 1969. The objectives of the Lymphoma Treatment Center include investigation of intensive chemotherapy programs in Burkitt's tumor, investigation of new agents, the study of central nervous system involvement, the obtaining of quantitative information on cellular kinetics, to establish a rational basis for immunotherapy, and to continue the study of delayed hypersensitivity to autologous tumor extracts. The Solid Tumor Center is designed to provide facilities to study hepatocellular carcinoma, particularly the correlation of serum alpha fetoprotein, histology and biochemical features, assay of serum prolinehydroxylase electronmicroscopy, studies of the metabolism by normal and tumorous liver of dichloromethotrexate, to search for the effectiveness of imidazole carboxamide (NSC 45388) in

melanoma patients, to assess host response and cutaneous reactivity to intradermal autologous tumor cell extracts, and to initiate preliminary studies of immunotherapy; in Kaposi's sarcoma, to continue a randomized study of chemotherapy in the benign and florid aspects, and to study tumor cell kinetics using colchemide.

Clinical Investigations:

A. Burkitt's Tumor

1. Of the 120 patients with Burkitt's tumor admitted over the past 3-1/2 years, the first 57 patients were treated initially with one or six doses of cyclophosphamide in a randomized trial. Subsequently, patients with generalized disease were treated either with multiple doses of cyclophosphamide or with a sequential cyclic regimen named TRIKE, consisting of cyclophosphamide, vincristine, methotrexate, and cytosine arabinoside. Results of the first trial have been published and the preliminary results of the second trial are summarized in the following table:

	No. Treated	No. Complete Remission	No. Relapse	No. Died
Cyclophosphamide	19	19	6	4
TRIKE	21	19	6	2

It appears that the introduction of the TRIKE regimen did not alter the pattern of relapse or the recurrence of neurologic involvement as compared to cyclophosphamide. Central nervous system involvement continues to be a major therapeutic problem in Burkitt's lymphoma although remissions can be easily achieved with a variety of agents. Recurrence of malignant pleocytosis and ultimate resistance to intrathecal chemotherapy occurs in the majority of patients, and no single regimen has produced remissions.

A randomized trial of prophylactic intrathecal chemotherapy in Stage I-III patients has shown no protective effect. Further therapeutic attempts will employ intrathecal cytosine arabinoside for three days followed by methotrexate on the fourth day with and without concomitant systemic hydroxyurea in a randomized trial. In addition, the Ommaya reservoir will be employed in patients refractory to the above regimens. A complete analysis of the clinical features and pathogenesis of central nervous system involvement by Burkitt's lymphoma has recently been published, and further results of intrathecal chemotherapy of malignant pleocytosis have been submitted for publication.

The survival analysis of 92 treated and evaluable Burkitt's lymphoma patients reveals that clinical staging on admission is still the best prognostic indicator available.

Stage	Site on Admission	No. Patients	Percent Surviving One Year	Percent Surviving Two Years
I-II	Localized (jaw)	26	92% (14)*	82% (7)
III	Visceral	47	67% (26)	64% (20)
IV	CNS or Marrow	19	52% (4)	- (0)

*Number in parenthesis indicates patients free of disease beyond one or two years from treatment.

2. Cell Kinetics

In collaboration with Professor O. Iverson of the University of Oslo (former visiting Professor of Pathology, Makerere Medical School), a study of tumor cell kinetics both in vitro and in vivo has shown that the growth fraction in Burkitt's lymphoma approaches 100%, and the potential doubling time is 22 hours. This information is consistent with results of other in vitro kinetic studies of cultured or biopsied Burkitt cells, indicate that Burkitt's lymphoma is the fastest growing human neoplasm, which may account for the extraordinary sensitivity of this tumor to chemotherapeutic agents. With precise kinetic information now at hand, rational treatment regimens aimed at total cell kill can be devised.

3. Immunology

Results of investigations on tumor-specific cell-mediated immunity in 30 evaluable patients, initiated in 1968 by Dr. Leroy Fass and continued by Dr. Avrum Bluming, have been submitted for publication. In brief, several important facets of tumor-specific cellular immunity to extracts of Burkitt's lymphoma have emerged. First, histologically confirmed delayed hypersensitivity reactions, observed in half of the patients tested during clinical remissions, were associated with more prolonged remissions than in the non-responding patients. Although the use of suitable controls has confirmed that positive responses are most likely directed against tumor-specific transplantation antigens, it is not clear at the present time whether these reactions represent an effective antitumor host response or passively reflect the absence of residual tumor. Second, there is no correlation between positive cutaneous reactivity to autologous tumor extracts and the presence of active central nervous system disease.

Other immunologic studies include projected investigation of cytotoxic antibody titers in Burkitt's lymphoma patients as well as the application of the macrophage migration inhibition technique to provide in vitro correlates to cellular immunity testing in Burkitt's lymphoma. Continued

studies of the immunoglobulin levels in Burkitt's lymphoma have confirmed the observation that IgM levels are significantly decreased and tend to remain low even in remission, although the significance of this phenomenon is still unclear.

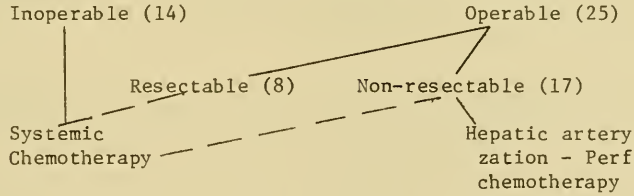
4. Cytogenetics

In Dr. Bluming's chromosome analysis of the tumor tissue of 13 evaluable Burkitt's lymphoma patients, the majority of preparations were euploid and normal in appearance. Abnormalities, when present, included marker chromosomes, breaks, and secondary constrictions that most often affect groups A and B but not group C as has been reported in cytogenetic studies of cultured Burkitt's lymphoma cells. Recurrent tumors are also being studied to determine whether these represent a recrudescence of the original clone of tumor cells or a new disease derived from a different cell clone.

B. Hepatocellular Carcinoma

1. Patient Evaluation

All patients referred to the Center with a suspected diagnosis of hepatocellular carcinoma were accepted for study and evaluated with colloidal gold liver scans, aortagrams, and determinations of alkaline phosphatase, alpha fetoprotein, proline hydroxylase, and Australia antigen, in addition to routine tests. Following these procedures the 39 patients studied were categorized as follows:



Most of those deemed inoperable were transferred to Mulago Hospital for protocol chemotherapy with bis-chloroethylnitrosourea (BCNU) under the supervision of Dr. C. Sezi and Dr. F. Saidkali of the Department of Medicine; others received either dichloromethotrexate (DCM) or a combination of bis-chloroethylnitrosourea (BCNU) and 5-fluorouracil (5-FU) at the Solid Tumor Center. Potentially operable patients were reviewed by the "hepatoma team" composed of the NIH investigators, the Mulago surgical team headed by Professor S. Kyalwazi, a radiologist, an anesthesiologist, and a coagulation specialist. At the time of exploratory laparotomy eight of the 25 patients were felt to have potentially resectable lesions. Of two long-term survivors who were treated with systemic chemotherapy, one relapsed on BCNU-5-FU and the other developed recurrent tumor following DCM.

2. Chemotherapy

Fourteen inoperable and three operable but non-resectable patients eligible for systemic chemotherapy received either DCM or a combination of BCNU or 5-FU. In addition, two patients received systemic chemotherapy as an adjunct to partial hepatic resection. To date, 23 courses of DCM, given to 13 patients in 4-hour infusions daily for a maximum of 10 days, have produced no objective antitumor response. Observations on myelosuppression following DCM revealed the following: (1) Daily doses of less than 15 mg/m^2 x 10 days were uniformly nontoxic, but moderate to severe myelosuppression occurred in cirrhotics given daily doses ranging from 15-25 mg/m^2; (2) Non-cirrhotic patients developed myelotoxicity with daily doses ranging from 25-35 mg/m^2; (3) Thrombocytopenia was not a clinically significant manifestation of DCM toxicity. Gastrointestinal toxicity, manifested by stomatitis and diarrhea, was observed at some time in 11 of the 13 patients.

The published results of systemic chemotherapy in hepatocellular carcinoma are not encouraging, however, there is some evidence that drugs given through a hepatic artery catheter can produce significant palliation and prolong survival in this disease. Although several technical problems have made a full program of infusion chemotherapy impractical at this time, four patients have had catheters placed in their hepatic arteries at the time of exploratory laparotomy. In two patients, catheter placement was inadequate (as assessed by post-operative angiography). The third patient died of unknown causes 48 hours post-operatively, and the fourth developed septicemia from an infected catheter tip. The hepatic artery catherization program will be re-evaluated and standardized.

Some objective responses in patients with hepatocellular carcinoma have been reported elsewhere following treatment with 5-day combination courses of BCNU (40 mg/m^2/day) and 5-FU (370 mg/m^2/day). At the Solid Tumor Center, 12 such courses were given to seven patients. Two of these patients had undergone partial hepatic resection and had no measurable evidence of residual tumor at the time chemotherapy was begun; one developed a subphrenic abscess and received only one course of treatment (exploration revealed multiple tumor recurrences in the residual portion of the right lobe of the liver); the other patient is not yet evaluable. None of the remaining five patients had an objective tumor response, and all died within three months of the start of treatment.

3. Alpha Fetoprotein (AFP)

A simple diagnostic test for the detection of occult hepatocellular carcinoma would be of tremendous clinical benefit. It is anticipated that AFP determination will prove to be just this. To date, approximately 500 sera have been tested for AFP. Of the 300 from pregnant women, normal controls, or patients with varied diagnoses other than

hepatoma, all were negative. Of 70 patients with cirrhosis and 30 with suspected viral hepatitis, only one, a patient with cirrhosis, had a positive test. Of 86 patients with biopsy proven or clinically suspected hepatocellular carcinoma, however, 59% or 69% were positive. Analysis of the results by age reveals that younger patients with hepatocellular carcinoma are almost uniformly positive, whereas the majority of negative tests were observed in patients above the age of 30.

4. Australia Antigen (Hepatitis-associated Antigen, HAA)

Although the cause of hepatocellular carcinoma remains unknown, most previous research has been on aflatoxin, a mold product known to cause hepatoma in laboratory animals. More recently, studies done at the Solid Tumor Center in cooperation with Drs. L. Barker and M. Peterson at the Division of Biologic Standards, NIH, have suggested some relationship between viral hepatitis and hepatocellular carcinoma.

Serum HAA in Liver Disease

	Total	Number Positive	Percent Positive
Controls			
Non-neoplastic	107	5	5
Neoplastic	55	0	0
Total	162		4
Hepatitis			
Proven	13	8	62
Suspected	10	4	40
Total	23	12	52
Cirrhosis			
Proven	40	10	25
Suspected	24	5	21
Total	64	15	23
Hepatoma			
Proven	44	16	36
Suspected	19	7	37
Total	63	23	37

5. Enzymology

Preliminary studies by Dr. H. Kaiser and coworkers of the National Heart Institute have indicated that proline hydroxylase, measurable in normal serum, was markedly elevated in a few patients with hepatocellular carcinoma. Elevated serum enzyme levels were observed in 72% of patients with hepatocellular carcinoma, 49% in cirrhosis, 33% in hepatitis, and 16% in controls. Because proline hydroxylase may be a tumor product, quantitation of this serum enzyme may yield a possible biochemical marker of disease activity, similar to AFP. Both of these tests are performed on serial specimens before and after surgery or chemotherapy with the aim of assessing their relative usefulness as indicators of residual tumor or response to treatment.

We have continued in vitro studies to determine the ability of human hepatocellular carcinoma tissue to metabolize dichloromethotrexate oxidase (DCM) to its inactive form. Preliminary results of qualitative testing, presented in last year's progress report were inconclusive because the test was not sensitive enough to detect the very low levels of DCM oxidase present in normal human liver. In recent assays tissue was incubated with DCM for varying periods of time and the supernate shipped to Dr. Johns at the NIH, who is performing quantitative tests to determine the ability of the supernate to inhibit hydrofolate reductase (an indirect measure of DCM inactivation).

C. Malignant Melanoma

1. Of 37 patients with malignant melanoma admitted during the past year, the primary sites of involvement were lower extremity (29), upper extremity (2), nasopharynx (3), and unknown primary (3). Eight of the 37 had vitiligo, often to a marked degree, occurring in most within one year of the development of the primary lesion or lymph node metastases. The nature of this phenomenon is under investigation.

Patients with widely disseminated or unresectable malignant melanoma are treated primarily with chemotherapy, although surgery is used as a palliative adjunct. Initial trials utilizing NSC 45388 (DTIC) were generally unsuccessful, although one dramatic but transient tumor response was seen among 12 evaluable patients. Those of the immunosuppressive potential of DTIC were also done during the clinical trials and the results of these studies are still under analysis.

Of 20 patients failing to respond to this regimen who were treated subsequently with a combination of BCNU and vincristine, seven are evaluable at the present time. One patient with cerebral metastases had dramatic clearing of the sensorium and regression of skin nodules after one course of treatment, but the response was short-lived, and she died after her first course of treatment with wide-spread disease.

The present plan is to complete the evaluation of the BCNU-vincristine combination and then employ other forms of chemotherapy. The next contemplated study is an evaluation first of hydroxyurea, then of cytosine arabinoside, and ultimately of both agents used in combination.

2. Immunotherapy.

Preliminary immunotherapy studies were designed to evaluate the effects of BCG (conventional intradermal versus dermal sacrification) and host immune responses prior to initiation of controlled, randomized immunotherapy trials. Acceptalbe for this phase of the study were all patients who had undergone resection of localized disease on an extremity and subsequent block dissection of regional lymph nodes.

3. Cytogenetics

Chromosomal preparations of human melanoma tissue show hyperploidy in all karyotypes evaluated, an observation consistent with other reports in the literature.

Honors and Awards: None

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Serial Number NCI 3425
1. Medicine Branch
2. NCI-VA Medical Oncology Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemotherapy of Cancer

Previous Serial Number: 3024

Principal Investigator: Oleg S. Selawry

Other Investigators: Patrick J. Creaven, Martin H. Cohen, Heine H. Hansen, Franco M. Muggia

Cooperating Units: The NCI-VA Medical Oncology Service is a cooperative project of the National Cancer Institute and the Veterans Administration. Funds are provided by the National Cancer Institute, administered by the Veterans Administration Central Office. Facilities are provided by the Washington Veterans Administration Hospital.

Man Years:
Total: 29
Professional: 18½
Other: 10½

Project Description:

Objectives:

1. Introduction of new anticancer agents into clinical trials and pharmacology of new antitumor agents.

2. Improvement in treatment of patients with bronchogenic carcinoma.

Summary of Major Developments:

1. The 30-bed Oncology Ward was officially opened in October, 1970. Present occupancy is 2/3 of capacity; full occupancy, including the two laminar air flow reverse isolation rooms, is planned for July 1, 1971.

2. Seven Phase I protocols originated and were in study in the Oncology Service during the past year. Three were completed.

3. The feasibility study on intensive chemotherapy of bronchogenic carcinoma showed that responders had more than twice the survival of nonresponders and that the groups of patients with poorest prognosis, including small cell carcinoma and adenocarcinoma, had the best response.

4. Recognition of tumor cells in the bone marrow in 50% of patients with small cell carcinoma represents an unexpected finding and indicates unexpectedly early spread of disease which would have been unnoticed in skeletal surveys.

Detailed Reports:

1. Clinical Research

1.1 Phase I Initial Clinical Pharmacology of New Antitumor Agents

The following studies originated from the NCI-VA Medical Oncology Service:

1.11 CCNU (1-(2-chloroethyl)3-cyclohexyl-1-nitrosourea) (NSC 79037): The Phase I study is completed, establishing 130 mg/m^2 q 6 weeks p.o. as the recommended dose for therapeutic trials. Objective response with more than 50% decrease of tumor size has been observed in 3/7 patients with bronchogenic carcinoma, 1/2 with pancreatic carcinoma, and 2/2 with lymphomas. Six of 8 patients with malignant brain tumors showed definite clinical improvement of 2 to 12 months duration.
Disposition: Phase I--Intensification of treatment with drug administration at intervals of 4 weeks is currently underway.
Phase II--A comparative therapeutic trial comparing CCNU with BCNU in patients with advanced Hodgkin's disease is conducted by this service in cooperation with the Acute Leukemia Group B. Fourteen valid patients are thus far included into each treatment program. Preliminary data show CCNU to be at least equal to BCNU. CCNU is used in several other Phase II studies by others.

References: 4.33, 5.5, 5.9

1.12 Camptothecin Sodium (NSC 100880): A total of 40 patients were entered during the past year. Two dose schedules have been explored. Fourteen patients received 21 courses of weekly intravenous camptothecin at dose levels of 20, 30, 44, and 67 mg/m^2. Reversible cystitis, thrombocytopenia, and leukopenia occurred at all dose levels; the latter was consistent at 67 mg/m^2 after a median of 2 doses and it occurred at the lower doses after a median of 3, 3, and 5 doses, respectively. Reticulocytopenia below 0.3% occurred in all 4 patients at 67 mg/m^2, and a decrease in hematocrit was seen in all 4 patients treated but one. Transient antitumor effect was noted in one patient with an adenocarcinoma of the stomach and one patient with an adenocarcinoma of the lung.
Drug tolerance to daily loading courses for 5 days was then explored beginning at 1.5 mg/m^2 total in view of the marked sensitivity to daily, as compared to weekly administration, in preclinical systems. At the highest level reached to date, 75 mg/m^2 total, we have observed leukopenia in 3/6 patients and reticulocytopenia in 3/4. One patient at 100 mg/m^2 total has shown thrombocytopenia, leukopenia, and cystitis. No evidence of antitumor effect was noted in the patients receiving loading courses. Dr. Patrick Creaven and

carried out pharmacologic studies on patients receiving the drug daily at the highest doses.
Disposition: The Phase I study with weekly doses is completed. Starting doses of 30 to 70 mg/m^2/week i.v. are recommended for therapeutic trials. Tolerance to 5-day courses is to be completed. Therapeutic trials are currently underway elsewhere.
References: 4.43

1.13 PTG (4' demethyl epipodophyllotoxin-B-D-thenylidene glycoside) (VM26, NSC 122819): During the past year 4 additional patients have been evaluated at a dose of 67 mg/m^2/week, eventually escalated to 90 mg/m^2/week if previous six-week courses were tolerated with minimal leukopenia. At 90 mg/m^2, leukopenia was again observed with no thrombocytopenia; some nausea and vomiting were also noted. A subjective response of 8 weeks duration was observed in a patient with astrocytoma Grade IV who was also on maintenance prednisone at the time.
Disposition: The Phase I trial is finished. A Phase II trial of PTG in combination with CCNU is considered in patients with brain tumors.
References: 4.41

1.14 Bleomycin (NSC 125066): Twenty-one patients from the Oncology Service and 27 from other cooperating units with advanced cancer included on the study since 4/16/70 received bleomycin twice weekly i.v. x 12. Three or more patients were entered per dose level at spaced intervals. Doses of 1.25 and 2.5 mg/m^2 x 12 were nontoxic. Nonprohibitive toxicity occurred at 4, 6, 8, and 11 mg/m^2, respectively, x 12. Prohibitive toxicity was noted in 1/7 patients on 15 mg/m^2, 0/5 on 20 mg/m^2, 2/3 on 26 mg/m^2, and 3/3 on 35 mg/m^2 at progressively shorter time intervals. Reversible complications included fever in 17 patients, alopecia in 15, dermatitis (pigmentation, swollen fingers and palms) in 14, oral ulcers in 6, leukopenia, thrombocytopenia or vomiting in 3 patients each. Bilateral pulmonary infiltrates occurred in 2 patients and were ascribed to bleomycin in 1 patient on 26 mg/m^2 x 10 to a total of 520 mg. Two additional patients had infiltrates after prolonged courses of 15 mg/m^2 and 20 mg/m^{2}to a total of 525 and 582 mg, improving slowly on prednisone. Objective 50% tumor regression occurred in 9 patients, including 4/28 squamous cell carcinoma, 4/4 lymphosarcoma, and 1/1 testicular embryonal cell carcinoma. Responses occurred at a median dose of 11 mg/m^2 within 1 month and continued for 2 to 5+ months. The unusual toxicological characteristics make bleomycin uniquely suitable for therapeutic trial in short courses alone or in combination with other agents.
Disposition: The pharmacology part of the study is closed to new entries. The therapeutic part of the study will remain open until it is superceded by disease-oriented therapeutic protocols.
References: 4.510

1.15 Methotrexate (NSC 740)-Leukovorin: Previously we have shown that leukovorin can be given up to 72 hours after methotrexate without further increase of toxicity when the ratio of methotrexate to leukovorin was

2:1. In an attempt to increase further the therapeutic effect without increasing toxicity, the dose of leukovorin has been decreased; at present, the ratio 2:0.3 is being tested. Thirteen patients have been treated with a total of 38 courses given. Objective responses were noted in 2/5 patients with squamous cell carcinoma of the head and neck.
Disposition: Study of methotrexate is continued for the pharmacologic study of the distribution of radioactive methotrexate.
References: 5.8

1.16 Isophosphamide (NSC 109742): A study protocol was designed and approved to explore the tolerance of patients with advanced cancer, to begin as the drug becomes available. The drug will be given intravenously every 3-4 weeks. Preliminary studies in Germany have shown isophosphamide to be superior to cyclophosphamide in the treatment of small cell anaplastic (and oat cell) carcinoma of the lung.

1.17 BPP (bis (3,5 dioxopiperazin-1-yl) propane): The Phase I study is being conducted in order to determine the toxic dose level for clinical use as a single dose, then in a weekly schedule.

1.2 Therapeutic Studies

1.21 Intensive Chemotherapy of Bronchogenic Carcinoma: This feasibility study was closed for new patient admissions in August, 1970, after inclusion of 48 consecutive patients, of which 46 are valid for evaluation. Twenty-seven patients had limited disease, 17 had extensive disease. The median survival as of 3/1/71 was 300+ days with 14 patients still alive in the group with limited disease versus 2/17 with extensive disease with median survival of 123 days. Patients with responsive disease lived significantly longer than nonresponders, 324+ days compared to 138 days in limited disease, and 205 days compared to 123 days in extensive disease, respectively. A total of 22 patients of 36 patients with evaluable or measurable disease responded to combined treatment. Patients with small cell carcinoma and adenocarcinoma had the highest response rate and longest survival, in contrast to patients with squamous cell or anaplastic carcinoma.
Disposition: The study is closed to new patients. A new study was designed for evaluation of comparative groups.
References: 4.32, 4.36, 4.56, 4.59, 4.512, 4.513, 4.514

1.22 Intensive Chemotherapy and Radiotherapy in Patients with Bronchogenic Carcinoma: This study originated during the past year as an expansion of the preliminary study above. Forty-one patients have been entered, 14 with limited disease and 27 with extensive disease. Random allocation of patients is carried out to compare radiotherapy alone with chemotherapy and radiotherapy in limited disease, and 2-drug combination with 3-drug combination in extensive disease. Patients are randomized and treated according to cell type. The following institutions are participating in this study: Mayo Clinic; Walter Reed

Army Medical Center, the University of Louisville Medical Center, Louisville, Kentucky; and the Groote Schuur Hospital, Capetown, South Africa.
Disposition: The study will continue.

1.23 Intensive Combined Chemotherapy of Testicular Tumors: Dr. Heine Hansen advanced a protocol for treatment of testicular tumors which led to a mutually satisfactory cooperative treatment program with the NCI, Walter Reed Army Medical Center, and the National Naval Medical Center. Because of limitations of patient accrual at this station, the protocol will be coordinated by the Oncology Section, Walter Reed Army Medical Center.

1.24 Study of Cholesteric Thermography: Two patients were studied during the past year. No untoward side effects were noted. The study is not being actively pursued at this time because of limitations of personnel.

1.3 Participation in Cooperative Clinical Studies

1.31 Protocol for the Study of the Intensive Combination Chemotherapy in the Treatment of Hodgkin's Disease (Solid Tumor Service, Medicine Branch, NCI): One patient was staged and entered on this study.

1.32 Dactinomycin and Mithramycin in Patients with Hypercalcemia, Phase I (ECOG #0270): One patient with laryngeal carcinoma and hypercalcemia was entered.

1.33 Comparison of CCNU and BCNU in the Treatment of Patients with Advanced Hodgkin's Disease, Phase III (ALGB #6953): One patient with Hodgkin's disease, stage IVB, was entered.

1.34 Treatment of Lymphosarcoma and Reticulum Cell Sarcoma (ALGB #7051): One patient with lymphosarcoma was entered.

1.35 Phase I Study of Intravenous Procarbazine (Solid Tumor Service, Medicine Branch, NCI): One patient with an astrocytoma, grade III, has been entered.

1.4 Related Research

1.41 Installation of two Litton laminar air flow units for reverse isolation was completed and the rooms are now ready for use.

1.42 Evaluation of lysozyme (LZM, muramidase) activity in patients prior to and during chemotherapy: Because plasma muramidase shows some correlation with granulocyte turnover, this determination has been explored primarily in patients with bronchogenic carcinoma. Plasma LZM in 25 patients prior to treatment did not differ significantly from normal. Levels declined parallel to the white cell count

following cyclophosphamide and methotrexate, but, following day 10, increased to normal or supernormal levels in spite of persistent granulocytopenia. In addition, patients manifesting the most severe granulocytopenia did not have expected low levels. These studies suggest that the granulocytopenia following cytotoxic agents is in part explained by increased utilization of granulocytes rather than just bone marrow hypoplasia.

References: 4.44

1.43 L1210 Experiments: For the past three years, studies have been done demonstrating enhancement of antitumor effect of BCNU by various "membrane active" drugs such as vitamin A, progesterone, and testosterone. More recently it was demonstrated that the addition of caffeine further enhances the therapeutic effect of the "membrane active" drug and caffeine. Studies are continuing in this area.

References: 4.21

1.44 Screening of hormone abnormalities in patients with bronchogenic carcinoma, such as gonadotropins, ketogenic steroids and ketosteroids and thyroid function studies, is being carried out in cooperation with Dr. Saul Rosen, Endocrinology Laboratory, NCI. Fifty-five patients have been evaluated; 3 patients were transferred to NIH for specific studies.

1.45 Evaluation of initial serum levels of zinc in patients with bronchogenic carcinoma, with follow-up during treatment is being done in cooperation with Dr. James G. Smith, Trace Elements Research, VA Hospital, Washington, D.C. The purpose of the study is exploration of the possible correlation to cell type and therapeutic response. Six patients have so far been evaluated.

2. Laboratory Activities

2.1 Biochemical Pharmacology Laboratory

During the past year the Biochemical Pharmacology Laboratory has expanded. The staff now includes the Chief, a pharmacologist, and a technician. Major equipment has been acquired, including a Packard 7201 radiochromatogram scanner, an Aminco-Bowman spectrophotofluorometer, and a LKB fraction collector. Studies have begun or continued on four durgs: methotrexate, camptothecin sodium, isophosphamide, and BPP (± bis (3,5 dioxopiperazin-1-yl) propane).

Methotrexate Studies: The current protocol ST 35, Methotrexate-Leukovorin, calls for eligible patients to receive 80 mg/m^2 of methotrexate at dose level 1, and for the dose to be increased by 50% steps to toxicity (dose level 2). The patients then receive double this dose (dose level 3), followed by leukovorin. It was proposed to study the plasma levels and distribution of methotrexate at the three dose levels by the use of (^{3}H) methotrexate to be infused over 5

minutes, serum samples collected at fixed intervals, and samples of tumor, bone marrow, and liver to be taken and assayed for radioactivity. So far four courses in three patients have been studied in this way. Solid samples are assayed after stabilization in NaOH and BBS3. The accrual rate is low because of the difficulty in obtaining regular supplies of the (^{3}H) labelled drug. Preliminary results indicate that the drug passes freely into the marrow space. It does not appear to be selectively accumulated by the liver, contrary to the predictions of the model of Oliverio and Zaharko (Oliverio, V., and Zaharko, D. Tissue distribution of folate antagonists. Ann. N.Y. Acad. Sci. In press). The study will continue and should be complete in 6 to 8 months.

Camptothecin Sodium: This drug is currently being studied in 5-day loading courses. It was of interest to determine the plasma half-life rate and degree of excretion of the drug and degree of accumulation. The previously existing method for fluorometric determination of camptothecin was considered too complex for routine use, so a simple method was developed to determine the $t_{\frac{1}{2}}$ of the distributional and excretory phases of the drug. Urinary excretion was also determined. Most of the patients so far studied do not show progressive accumulation of drug in the plasma over the 5-day period. However, less than 50% of the dose in each case was recovered in the urine and no drug was found in the feces. Extensive breakdown to non-fluorescent material or retention in the body for long periods might explain the unaccounted-for drug. The drug is extensively bound to plasma proteins and this aspect has been investigated by electrophoretic separation of plasma proteins, centrifugal filtration, and equilibrium dialysis and fluorometric titration studies in vitro. These studies will be expanded. A definitive pharmacokinetic study of the drug will be conducted shortly for measurement of full and total camptothecin levels.

Isophosphamide: This drug will enter clinical trials shortly. It has not yet been used in humans in this country. Our investigations have, therefore, been confined to studies in in vitro systems. Plots of metabolism versus time have been made and compared with similar plots for cyclophosphamide. Studies are in progress to determine the effect of pretreatment with phenobarbital on the metabolism of isophosphamide. V_{max}, K_m cofactor requirements, inhibitor effects, and other studies are also in progress on this system. Thin layer chromatographic separations of isophosphamide microsomal metabolites are also being carried out. As (^{14}C) isophosphamide becomes available, these studies will be expanded.

BPP (±bis (3,5 dioxopiperazin-1-yl) propane): This drug has recently become available to use and investigations have commenced on thin layer chromatography metabolism of the drug in tissue homogenates and reaction with DNA. So far, only preliminary results have been obtained.

A research study for investigation of the effect of antineoplastic agents on the levels of enzymes of drug metabolism has begun. Test systems will determine the effects of treatment with new antineoplastic agents being studied in this unit on the activities of liver microsomal enzymes and the extent to which the metabolism of antineoplastic agents is altered by pretreatment with other medications which patients commonly received on this unit such as pain, sleep, and anti-nausea medications.

2.2 Cell Kinetics Laboratory

The aim of cell kinetic studies at present is to obtain data on the labelling index, DNA synthetic time (t_s), generation time, and growth fraction in patients with untreated bronchogenic carcinoma, and to correlate these with various cell types and prognostic parameters. A technique of intramarrow labelling, modified from Goddell, Young, and DeVita (Proc. Amer. Assoc. Cancer Res. 11:31, 1970), was developed because of the propensity of small cell carcinoma to invade the posterior iliac marrow. Nine patients with a variety of normal and abnormal marrows were subjected to bone marrow aspirates and biopsies following 100 microcuries of ^3H thymidine. Adequate label was generally seen in both marrow aspirates and biopsies throughout the specimen. With this background, the same technique was applied in patients with untreated bronchogenic carcinoma. Five patients have been studied by the double label intramarrow technique. Thus far, the DNA synthetic time of 10 hours is available for one patient. The labelling indéces in two of these patients and one other patient studied by the single label intramarrow method were 18.6, 18.2, and 15.8%, respectively. In the event of suitable patients, plans have been made to study the growth fraction following intravenous loading with ^3H thymidine, as well as labelled mitosis studies; this would allow determination of all cell kinetic parameters. The labelled mitosis technique is applied at present to study skin turnover in a patient with mycosis fungoides in order to gain experience with the use of the technique of intralesional ^3H thymidine injection in other solid tumors. In addition, studies of primary lung tumors subjected to thoracotomy are planned to answer questions regarding differences in cell population kinetics between primary and metastatic sites. Techniques are also being applied that may eventually allow serial determination of labelling indeces following chemotherapeutic agents.

References: 4.42, 4.47

2.3 Cell Culture Laboratory

The spin filter culture method of Thayer and Himmelfarb is being set up with modifications to permit re-utilization of tissue culture media. It is planned to study drug scheduling in this system so that combinations of drugs may be used in more optimal fashion. Kinetic study will be done during the treatment regimen to predict and later correlate survival data with kinetic data.

2.4 Hematology Laboratory

The following tests were performed in the Oncology Hematology Laboratory during the time period 4/1/70 to 4/1/71:

Bone marrow aspirations	155
Bone marrow biopsies	155
Differentials	744
Reticulocytes	1204
Platelets	1343
Hematocrit*	529
WBC*	502
MCV	29
Size and cell distribution of RBC	29
Size and cell distribution of platelets	29
Zinc levels	10
Hematoxylin and eosin stains	5
Fe stain	1

*The Model S coulter counter, provided by the Oncology Service, was put into use in the VA Hospital Clinical Laboratory in June, 1970, and has been used for WBC and hematocrit tests on Oncology patients.

2.5 Bacteriology Laboratory

The following studies have been initiated during the past year:

Radiological assay for dihydrofolate reductase: The objective of this study is the development of a sensitive radiological method for the quantitative determination of dihydrofolate reductase, an enzyme involved in thymidylate synthesis which occurs in cell nuclei and therefore might be present in significantly higher amounts in tumors. Since methotrexate is a potent inhibitor of dihydrofolate reductase and since this drug-enzyme relationship is stoichiometric, development of a precise enzymatic assay for any of the 4-amino analogs of folic acid is possible. More than 80 scintilation counter readings were performed in the development of the test, using extract of guinea pig kidney as the enzyme and generally labelled folate as the isotope source. Standardization of the test was attempted, but not attained, with the Bratton-Marshall test. Twenty-five determinations were made. Since it was difficult to obtain a supernatant fluid free of charcoal, it is likely that failure of standardization was due to the charcoal. Five brands of charcoal were tested with little or no improvement over the original. The study was discontinued in October, 1970.

Development of a sensitive biological assay for methotrexate: Because methotrexate is a potent inhibitor of dihydrofolate reductase, it was felt that the turbidimetric folic acid assay technique could be applied to the quantitative determination of methotrexate. The minimum inhibitory concentration of this drug for L. actobacillus casei was determined in various media and at various folate concentrations.

Under the most propitious conditions, L. casei is sensitive to as little as 0.02 ng. This would enable as little as 0.04 ng to be detected in serum, accounting for at least a 1:2 dilution of the serum in the first tube. However, since the plate assay does not suffer from the disadvantages of tube assays, it was decided to develop a plate assay for methotrexate. Using L. casei in a cup-plate method similar to a penicillin assay method, it became established, after about 80 determinations, that by this method under the proper conditions, methotrexate can be accurately measured when present at as little as 0.5 ng/ml of serum. Further efforts are being made to increase the sensitivity of the test by testing various diluents which may allow greater diffusion of the drug into the agar.

The protected environment as a means of decreasing the number and severity of infections in treatment of cancer by chemotherapy: A study is being undertaken to determine the value of a protected environment in the reduction of infections in patients requiring intensive chemotherapy for bronchogenic carcinoma. After delays due to technical difficulties, the two laminar flow reverse isolation rooms are ready for occupancy. When adequate nursing staff are available, the study can begin.

The study protocol has been completed and work orders are being written. The patient and the environment will be kept under constant microbiological surveillance after the initial efforts to free the patient and the room of microorganisms, as far as possible. Microbiological analyses have been performed on articles such as utensils, linens, towels, disposable paper products, etc. Various methods have been tried for evaluation of techniques.

As an adjunct to the study, the bacteriological and fungal flora of the airways for patients with bronchogenic carcinoma will be investigated. It must be determined whether there is any difference in the flora of the bronchi with the size and location of tumors and whether lack of contact with bacteria by the protected environment alters or reduces the number and variety of organisms in the sputum or bronchi. Techniques will include aerobic and anaerobic cultures of sputum and bronchial secretions. Secretions will be obtained by a swab technique during bronchoscopy.

3. Additional Activities of the NCI-VA Medical Oncology Service

3.1 Clinical Procedures

3.11 Bone marrow biopsy and aspiration: The value of performing bone marrow biopsies and aspirations has been established, showing a hield of positive marrows with invasion of tumor cells in 50% of patients with small cell carcinoma (9/18 patients) versus a low yield in other tumor types (2/30 with anaplastic carcinoma, 2/34 with squamous cell carcinoma, and 1/15 with adenocarcinoma).

References: 4.35, 5.6

3.12 Bronchoscopy: An extensive program of bronchoscopy is planned in patients with bronchogenic carcinoma. The purpose is two-fold: 1. to serially follow tumor cell cytology in order to predict, if possible, by the cytologic changes, when tumor cells become resistant to a particular form of chemotherapy; 2. to employ bronchoscopy to obtain adequate sputum samples for bacteriology to predict, if possible, the infecting organisms when a patient subsequently becomes septic.

3.13 Peritoneoscopy: Peritoneoscopy has been initiated for the purpose of staging of patients with bronchogenic carcinoma, in view of a study reporting 15% liver metastases at laparotomy in patients in whom thoracotomy was being contemplated (Bell, J.W. Abdominal exploration in one-humdred lung carcinoma suspects prior to thoracotomy. Ann. Surg., 167:199-203, 1968). Eleven patients have been staged to date: one had demonstrable tumor in the liver grossly and on biopsy; one other patient, subsequently declared extensive on bone marrow aspiration, had suspicious nodules visualized in the liver. Incidental findings were common: several patients had cirrhosis, one had cholelithiasis, and one, who had bronchogenic carcinoma complicated by pulmonary tuberculosis and an elevated alkaline phosphatase, was found to have miliary tuberculous granulomas on liver biopsy. Peritoneoscopy was also performed in four additional patients with other indications for the procedure (obstructive jaundice in a patient with small cell carcinoma; staging of a patient with untreated Hodgkin's disease; evaluation of hepatomegaly and ascites in a patient with tongue carcinoma; and evaluation of hepatomegaly in a patient with bronchogenic carcinoma and probable methotrexate hepatotoxicity).
The procedures have been carried out by Dr. Frank Muggia under the supervision of Dr. Nelson Trujillo, Consultant to the NCI-VA Medical Oncology Service.

3.2 Consultations

A total (projected from 4/1/71) of 305 consultations was performed by the Oncology medical staff in the Washington VA Hospital. This is a 50% increase over the previous year. In addition, numerous phone calls by outside physicians concerning chemotherapy were answered.

3.3 Nursing (Ward and Ambulatory Care Clinic)

From July 1, 1970 to January 1, 1971, there were 10 to 15 patients on the Oncology Ward. Since January 1, 1971, the unit has had an average of 21 patients. Currently, sixteen nursing staff members cover the ward and the Ambulatory Care Clinic (11 RN's, 3 LPN's, 2 NA's). Full ward occupancy is delayed by shortage of nursing staff; when the nursing staff reaches the planned total of 41, the ward, including the two

laminar air flow reverse isolation tooms, and the Ambulatory Care Clinic can operate at full capacity.
One hundred and five patients were transferred into the Oncology Ward during the year; 25 patients were admitted directly, totaling 130 patients cared for as inpatients during the year.
From July 1, 1970 through April 7, 1971, there have been 46 deaths. Eighty-six patients are presently followed by the Service: 17 inpatients on the Oncology Ward, 13 inpatients on other wards in the VA Hospital, and 56 out-patients. An average of 183+ out-patient visits per month were made to the Ambulatory Care Clinic; a total of 2,200 visits for the year.

3.4 Social Work Service

The Oncology Service Social Worker makes contact with all patients admitted to the Oncology Ward, with priorities established by referrals made by the medical staff, families and/or patient requests. In addition, she sees patients and families who visit the Ambulatory Care Clinic. The patients are followed as long as they are seen by the medical staff. Patients with bronchogenic carcinoma are followed until they are off study. The average caseload per month is 23+ inpatients and 29 out-patients.
A social work study was initiated during the past year: "A Social Survey of Patients on ST 53 Bronchogenic Protocol." The objectives of the study include obtaining data in order to: evaluate social status of patients, define primary social problems, establish a tentative hierarchy of problems, determine resources, determine mode of introduction to the social worker, describe social work services provided during the course of treatment.
An interview schedule is used for the initial interview of patients. Patients are followed throughout the course of treatment and contact with families is initiated and maintained. Some interim findings are:
Educational range: Grade 4 through 4 years college
Average grade achieved: 9.3
Income: range: 0-$916 per month; sixty-four per cent of the patients have an income less than $300 monthly; the remainder (with one exception) receives income below $500.
Only 7 patients (of 41) listed financial problems as their most serious problem. The majority of patients stated they knew what they were being treated for. The depth of their understanding is not known.

3.5 Educational Activities

Medical staff members attended and participated in regularly scheduled discussion meetings, rounds, and Tumor Board at the VA Hospital and the NCI. Informal discussions concerning research problems and developments in the field of oncology and, specifically, in the Oncology Service were held frequently. Staff physicians contributed 8 months of attending coverage on the VA Hospital medical wards. The Oncology Training Program has been planned and will begin July 1, 1971. Two

fellows of the Cancer Therapy Evaluation Branch of the NCI rotated through the Oncology SErvice from January through March and April through June.
Laboratory technicians attended lectures and seminars relating to their work at the VA Hospital.
The social worker participated in the VA Hospital social service orientation program for students and nurses. She attended the comprehensive Oncology rounds held weekly.
Educational activities of the nursing staff during the past year related to care of in- and out-patients. A major focus was on preparing for the utisization of the laminar air flow units. Over 50 in-service programs were conducted. Nurses participated in conferences and workshops of the American Cancer Society and the National Institutes of Health. Members of the nursing staff were participant-observers of the laminar air flow units in the USPHS Hospital, Baltimore, Maryland and at the Clinical Center, National Institutes of Health, Bethesda, Maryland. Daily patient rounds with the physicians have served as excellent in-service training for the staff nurses. The Head Nurse attends the weekly comprehensive Oncology rounds.
A series of talks were initiated in the Oncology Service in order that all Oncology employees might be informed on work in progress in the different areas of the Oncology Research and Clinical Program. A representative of each laboratory, study, and supporting service is scheduled to present objectives and current work in relation to the overall objectives of the Oncology Service.

3.6 Training and Workshops

J. Fedorko, Senior Bacteriologist attended a course, "Animals in Medical Research," VA Hospital, Washington, D.C.
S. Oster, Medical Technician, completed a USDA Graduate School course, "Fundamentals of Microbiology," and is now attending a USDA course, "Fundamentals of Medical Microbiology."
J. Bohan, Head Nurse, participated in a 30-hour workshop in leadership at the VA HOspital. The staff nurses completed a 4-week program in Respiratory Care, given by Dr. Scott, VA Hospital, Washington, D.C.
A. Reuschel, Social Worker, J. Bohan, Head Nurse, and M. Poblet, Staff Nurse, attended the Cancer Care Symposium in New York, N.Y., 10/15/70.
P.J. Creaven, M.D. participated in an invitational workshop on "Principles of Drug Evaluation in Man," University of Kansas Medical Center, Kansas City, Kansas, 6/19/70.

4. Related Activities

This section contains formal lectures with published abstracts, lectures without published abstracts, and participations in cooperative study groups, boards, and committees.

4.1 Patrick J. Creaven, M.D., Ph.D.

4.11 Member, NCI Operations Committee
4.12 Member, Decision Network Committee

4.2 Martin H. Cohen, M.D.

4.21 "Enhancement of the Antitumor Effect of BCNU by Vitamin A and Caffeine." Presented at the annual meeting of the American Association for Cancer Research, Chicago, Illinois, April, 1971. Proc. Amer. Ass. Cancer Res. 12:69, 1971. (Abstract)

4.22 Ziegler, J.L., Cohen, M.H., and Hutt, M.S.R.: Immunologic Studies in the Tropical Spenomegaly Syndrome in Uganda. East African Med. J. In press.

4.23 Admitted to membership in the American Association for Cancer Research and the American Society of Clinical Oncology

4.3 Heine H. Hansen, M.D.

4.31 Hansen, H.H., Selawry, O.S., and Andrews, J.R.: Treatment of Unresectable Bronchogenic Carcinoma with Combination Chemotherapy and Radiotherapy. Presented at the annual meeting of the American Association for Cancer Research, Chicago, Illinois, April, 1971. Amer. Ass. Cancer Res. 12:80, 1971. (Abstract)

4.32 "CCNU" Presented at the Phase I-II Liaison Meeting, National Cancer Institute, Bethesda, Maryland, November 13, 1970.

4.33 "Recent Developments in the Treatment by Chemotherapy of Solid Tumors." Presented at the Kiakonisse Hospital, Copenhagen, Denmark, September 12, 1970.

4.34 Hansen, H.H., Rizzo, C., and Muggia, F.M. Correlation of Cell Type and Bone Metastases in 75 Consecutive Patients with Lung Cancer. Proc. Amer. Soc. Clin. Oncology, April, 1971, No. 28. (Abstract)

4.35 "Staging and Treatment of Patients with Bronchogenic Carcinoma." Presented at the Subspecialty Conference, VA Hospital, Washington, D.C., March 1, 1971.

4.36 Admitted to membership in: American Association for Cancer Research, American Society of Clinical Oncology, American Society of Hematology, Eastern Cooperative Oncology Group

4.4 Franco M. Muggia, M.D.

4.41 "Phase I Study of 4' Demethyl Epipodophyllotoxin-B-D-Thenylidene Glycoside." Presented at the Phase I-II Liaison Meeting, National Cancer Institute, Bethesda, Maryland, November, 13, 1971.

4.42 "Implications of the Biology of Cancer Cell Growth." Presented at the Nursing Staff Conference, VA Hospital, Washington, D.C., January 12 and 18, 1971.

4.43 "Exploration of Two Dose Schedules of Camptothecin Sodium in Man." Presented at the meeting of the American Association for Cancer Research. Proc. Amer. Ass. Cancer Res. 12:41, 1971.

4.44 Muggia, F.M., Oster, S., and Hansen, H.H.: Correlation of Plasma Lysozyme and White Cell Count in Drug-Induced Leukopenia. Amer. Soc. Hematol. 13:96, 1970. (Abstract)

4.45 "Cell Kinetic Studies of Human Tumors." Presented at the Roger Williams Hospital Medical Seminar, Providence, Rhode Island, March 11, 1971.

4.46 "Cell Kinetics and Chemotherapy." Presented at the Subspecialty Conference, VA Hospital, Washington, D.C., May 3, 1971.

4.47 Admitted to membership in: American Association for Cancer Research, American Society of Clinical Oncology, American Colleg of Physicians, American Society of Hematology

4.5 Oleg S. Selawry, M.D.

4.51 "Prospects of Chemotherapy of Oat Cell Carcinoma." Presented at the Scientific Advisory Council, NCI, Bethesda, Maryland, September 29, 1970.

4.52 "Considerations for Early Treatment of Lung Cancer in Uranium Miners." Presented at Grand Junction, Colorado, October 12, 1970.

4.53 "Cyclophosphamide." Presented at the Chemotherapy Advisory Committee, NCI, Bethesda, Maryland, October 27, 1970.

4.54 "The Modified Fibonacci Search Scheme Approach to Phase I Study." Presented at the Phase I-II Liaison Meeting, NCI, Bethesda, Maryland, November 13, 1970.

4.55 Moderator, Panel Discussion on Phase I-II Protocols, Symposium on Statistical Aspects of Protocol Design, San Juan, Puerto Rico, December 10, 1970.

4.56 "Lung Cancer." Presented at the Subspecialty Conference, VA Hospital, Washington, D.C., January 27, 1971.

4.57 "Clinical Trials." Presented at the Oncology Seminar, National Cancer Institute, Bethesda, Maryland, February 3, 1971.

4.58 "Oncology Service Chemotherapy Program." Presented at the Social Workers Staff Meeting, VA Hospital, Washington, D.C., February 24, 1971.

4.59 "Prospects for Chemotherapy of Lung Cancer." Presented at the Staff Seminar, Mayo Foundation, Rochester, Minnesota, February 29, 1971.

4.510 "Bleomycin: Tolerance of Cancer Patients to Twice Weekly Dosage." Presented at the annual meeting of the American Association for Cancer Research, Chicago, Illinois, April 9, 1971. Selawry, O.S., Ohnuma, T., and Shedd, D.P. Proc. Amer. Ass. Cancer Res. 12:70, 1971. (Abstract)

4.511 "Cancer Chemotherapy: Choice of New Agents and Methodology of Initial Clinical Evaluation." Presented at the Research and Education Lecture Series, VA Hospital, Washington, D.C., April 15, 1971.

4.512 "Current Approaches to the Therapy of Small Cell Carcinoma." Presented at the Lung Seminar, VA Hospital, Washington, D.C., April 20, 1971.

4.513 "Differential Therapy of Bronchogenic Carcinoma." Presented at the Staff Conference, Roswell Park Memorial Institute, Buffalo, New York, April 28, 1971.

4.514 "Perspectives and Prospects in Chemotherapy of Lung Cancer." Presented at the Educational Cancer Program, University of Alberta, Edmonton, Canada, June 19, 1971.

4.515 Site Visits:
Clarksburg VA Hospital, Clarksburg, West Virginia
Grand Junction, Colorado
VA Hospital, Martinsburg, West Virginia
University of Louisville Medical Center, Louisville, Kentucky
Mayo Clinic, Rochester, Minnesota
Johns Hopkins Hospital, Baltimore, Maryland
Hahneman Medical College Hospital and Pennsylvania Hospital, Philadelphia, Pennsylvania

4.516 Membership and participation:
American Association for Cancer Research
American Society of Clinical Oncology
NCI Operations Committee
NCI Decision Network Committee
NCI Experimental Design Committee
Subcommittee on Care and Chemotherapy of Cancer Patients, Regional Medical Program
Veterans Administration Lung Group

4.517 Executive Secretary, Eastern Cooperative Oncology Group

4.518 Appointed Associate Professor, Department of Medicine, George Washington University Medical Center Department of Medicine, Washington, D.C.

5. Publications

5.1 Carbone, P., Frost, J., Feinstein, A., Higgins, G., Jr., and Selawry, O.: Lung cancer: Perspectives and prospects. An edited transcription of a combined clinical staff conference at the Clinical Center, NIH, Bethesda, Md. Ann. Int. Med. 73:1003-1024, 1970.

5.2 Chua, D.T., Veenema, R.J., Muggia, F.M., et al: Acid phosphatase levels in bone marrow: Value in detecting early bone marrow metastasis from carcinoma of the prostate. J. Urol. 103:462-466, 1970.

5.3 Cohen, M.H., Hirshaut, Y., Stevens, D., Hull, E.W., Davis, J.H., and Carbone, P.P.: Infectious mononucleosis followed by Burkitt's tumor. Ann. Int. Med. 73:591-593, 1970.

5.4 Ennis, H.L., Moller, L., Wang, J.J., and Selawry, O.S.: 2,3-dehydro-1-H-imidazo (1,2-G) pyrazole: A new inhibitor of deoxyribonucleic acid synthesis. Biochem. Pharmacol. In press.

5.5 Hansen, H.H., and Muggia, F.M.: Treatment of malignant brain tumors with nitrosoureas. Cancer Chemother. Rep. In press.

5.6 Hansen, H.H., and Muggia, F.M.: Detection of early bone marrow invasion. New Eng. J. Med. In press.

5.7 Hansen, H.H., Muggia, F.M., and Saini, N.: Cyclophosphamide and urinary bladder toxicity. New Eng. J. Med. In press.

5.8 Hansen, H.H., Selawry, O.S., Holland, J.F., and McCall, C.B.: The variability of individual tolerance to methotrexate in cancer patients. Brit. J. Cancer. In press.

5.9 Hansen, H.H., Selawry, O.S., Muggia, F.M., and Walker, M.S.: Clinical studies with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, NSC 79037). Cancer Res. 31:223-227, 1971.

5.10 Keyes, J.W., Jr., Selawry, O.S., and Hansen, H.H.: initial clinical trials of dibromodulcitol in patients with advanced cancer. Cancer Chemother. Rep. In press.

5.11 Muggia, F.M., Ghossein, N.A., and Hanok, A.: Creatinine phosphokinase and other serum enzymes during radiotherapy. J.A.M.A. 211:1345-1350, 1970.

5.12 Muggia, F.M., and Heinemann, H.O.: Hypercalcemia associated with neoplastic disease. Ann. Int. Med. 73:281-290, 1970.

Serial No. NCI-3426
1. Medicine Branch
2. Leukemia Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Hemorrhagic Complications of Acute Leukemia

Previous Serial Number: 3401 (being abolished)

Principal Investigators: Ronald A. Yankee, M.D., and Edward S. Henderson, M.D.

Other Investigators: Roger Halterman, M.D., Brigid Leventhal, M.D., Kenneth Graff, M.D., Barbara Henderson, M.D., Harvey Gralnick, M.D., and Paul Schmidt, M.D.

Cooperating Units: Blood Bank, Clinical Center
American National Red Cross, Washington, D.C.
Central Blood Service, Baltimore, Maryland
DCRT (NIH)
Immunology Branch, NCI
Microbiological Laboratories, Bethesda, Maryland
University Los Angeles, Los Angeles, California

Man Years:

Total: 14
Professional: 8
Others: 6

Project Description:

Objectives:

To improve methods of preventing or controlling thrombocytopenic hemorrhage in patients with leukemia and other malignancies.

Major Findings:

I. Long Term Platelet Support: We have continued our platelet transfusion studies in patients with aplastic anemia using platelets obtained repetitively from HL-A matched sibs. Three patients received eight units of platelets per week from sib donors for 2½, 3, and 4½ years. One patient expired from pseudomonas sepsis (2½ years). Responses remain excellent in the other two patients despite refractoriness to all other platelet donors. Of particular interest in these patients are studies performed for the purpose of detecting alloimmunization to sibling cells, i.e., immunization against non-HL-A antigens. Following one year of transfusion therapy, the

reactivity of patient lymphocytes against sib lymphocytes in mixed lymphocyte culture remains within the HL-A identical sib range. Granulocyte survival of 32P labeled HL-A identical sib granulocytes was found to be within the normal range. The cell cytotoxicity assay in which ^{51}Cr labeled donor lymphocytes are used as target cells during short term incubation with recipient lymphocytes is the only test that demonstrates reactivity consistent with low level sensitization. To date, humoral antibody has not been detected against HL-A sib donor lymphocytes platelets, or granulocytes.

Conclusions: These studies (1) establish the importance of determining HL-A genotypes in families of patients requiring long-term support; (2) they provide impressive evidence (2 patients) that the relationship of granulocyte compatibility to the genetic determinants of HL-A antigens is the same as that observed with platelets and lymphocytes and finally, (3) that the direct lymphocyte cytotoxicity assay may be a highly useful test for detecting evidence of recipient recognition of HL-A matched donor cells.

II. Selection of Compatible Donors by HL-A Typing: Although platelet compatibility can be ascertained by HL-A genotype analysis of family members, it has not yet been shown that typing and matching of HL-A antigens from non-related individuals is of value in the selection of compatible platelet donors. Our preliminary studies suggest, that as predicted, the search for compatibility, will require a large donor population (probably greater than 1,000 individuals). A computer search for specific HL-A phenotypes by Dr. Terasaki's and our own data bank has been used to select potentially compatible donors. Although good platelet increments have been observed with refractory patients, single transfusions of platelets from these selected donors, second or third transfusions have inevitably been accompanied by reactions and lack of response. The lymphocyte cross match test (testing the presence in recipient serum of antibodies against donor lymphocytes) lacks sensitivity as does the platelet complement fixation test, to detect this type of alloimmunization. The serum from one patient, for example was found to be cytotoxic to 60% of donors tested. However, when platelets from eight of the negative cross match donors were transfused, there was no evidence of response to any of the transfusions at a time when this patient was responsive to matched sibling's platelets.

These studies emphasize the need for development of better methods for recognizing HL-A antigens before attempting selection of non-related donors by HL-A phenotype. In addition, the insensitivity of our present means of detecting antibodies in recipients is further emphasized. It is clear that measurement of the platelet increment following transfusion may be used as an extremely valuable indicator of circulating antibody to HL-A antigens. Such alloimmunization can only be established, however,in the proper setting whereby other non-specific factors affecting platelets are excluded by demonstrable responsiveness to matched sibling platelets.

III. Prevention of Alloimmunization and Single Donor Support: The development of refractoriness to multiple transfusion of platelets from random donors

in non-immunosuppressed recipients occurs generally within two months. The response to platelets once resistance develops is extremely poor. It is not known whether transfusions of platelets obtained repetitively from individual donors can provide more prolonged periods of support than repetitive transfusions of multiple donor platelets. Only one patient with aplastic anemia is thus far evaluable. This patient has received 8 units of platelets per week from a single non-related randomly selected donor. Resistance and specific antibody (HL-A 12) against the donor platelets has occurred at 9 weeks. A new donor with a differing HL-A antigen phenotype has been selected and platelets transfused. Response both to this selected donor as well as to HL-A sib matched platelets are currently being studied.

In conjunction with this study, an attempt has been made to prevent alloimmunization to leukocytes in transfused blood by leukopak and buffy coat removal procedures. The presence in the recipient of a single specific antibody (HL-A 12) of donor types attests to the success of this program.

IV. Non-thrombocytopenic bleeding: Non-thrombocytopenic bleeding is a common complication of acute leukemia, particularly during the initial week of observation and treatment. The most common cause is disease-related disseminated intravascular coagulation (DIC), but less common causes determined by studies carried out in conjunction with the Clinical Center and the James F. Mitchell Foundation are: 1) isolated clotting factor deficiencies (X,V), and 2) primary fibrinolytic activator production. Both of these conditions have been shown to occur only during relapse, and in the fibrinolytic activator has been shown to be a product of leukemic myelomonoblasts. Precise differential diagnosis of hemorrhagic states is mandatory since therapies for the different coagulopathies are mutually antagonistic. Initial studies to determine that the effect of transfusion reactions on coagulation factors suggest a clinical picture of transient disseminated intravascular coagulation following platelet or transfusion. The resulting profound leukopenia (both granulocytes and lymphocytes) for periods up to 40 hours may predispose already leukopenic individuals to infection.

V. Selection of Compatible Platelets From a Population of Donors Enrolled in a Repetitive Plateletpheresis Program: In cooperation with and as a part of our contract, the Central Blood Service in Baltimore has established a plateletpheresis program. Each donor taking part in this program is given a special identifying code number and undergoes a 2-unit plateletpheresis twice weekly. At the time of transfusion at NCI, the platelet donor code number is recorded on a computer as well as on the patient's transfusion record. The response to transfusion is measured by use of the formula INC x m^2/unit (performed @ 1 or 2 hrs.) Subsequent transfusions are given on the basis of this response, i.e., if there is no response, a new donor(s) is empiracally selected; if a good response occurs then, the same donor platelets are transfused. For adequate platelet support, usually 2 or 3 donors (4 to 6 units) are required for each transfusion, given twice weekly.

During the past four months, three patients who exhibited partial resistance have shown improved responses to platelets using this method of donor selection.

As a part of the program, and in cooperation with CBS, all donors have now been typed by our laboratory for HL-A antigens. A computer program is currently being established with DCRT for correlation of transfusion response with donor and recipient HL-A antigen types. It is expected that this program will be extremely helpful in establishing (1) whether or not HL-A typing is important for selecting compatible platelet donors from the random population and (2) whether individual HL-A antigens can be classified as "strong" or "weak" antigens with respect to the development alloimmunization. It can be concluded from these preliminary studies that a systematic selection of platelet donors, even empirically, improves overall platelet support of patients with leukemia.

VI. HL-A Typing of Patients, Their Families, and Normal Donors: Histocompatability testing provides the framework for clinical studies on improving methods of patient support and basic investigations in tumor immunology.

Over 5,000 typings have been performed under contract with Microbiological Laboratories, Bethesda, Maryland and Dr. Paul Terasaki, Laboratory, Los Angeles, California. This includes 100 patient families and approximately 2,000 normal donors.

In animals, susceptibility to certain diseases has been related to the histocompatibility antigen type of the host. Similar relationships have been suggested by investigations in patients with leukemia, Hodgkin's disease, and lupus erythematosis. In our population of patients with acute lymphocytic leukemia (59 families) there appears to be a higher frequency of HL-A 2 when compared with non-leukemic families.

Further studies will be required to attach significance to these findings.

Proposed Course of Project:

1) Determination of the relative effectiveness of platelets from HL-A matched donors and from the random donor population.

2) Development of platelet complement fixation test as a supplement to typing by the lymphocyte cytotoxicity test.

3) Evaluation of HL-A antigen frequencies in patients with malignancy.

4) Study of the incidence of disease transmission from a repetitive platelet donor population (commercial source) compared to single unit platelet donors (volunteer source).

5) Evaluation of factors affecting platelet response including disseminated

intravascular coagulation associated with transfusion reaction.

Honors and Awards: None

Publications:

1) Perry, S. and Yankee, R.A.: Transfusion and preservation of platelets. In Johnson, S., ed. The Circulating Platelet. The Academic Press, Inc., New York and London. 1970.

2) Yankee, R.A.: The importance of histocompatibility. In Platelet Transfusion Therapy. Vox Sang (in press).

3) Gralnick, H.R., and Henderson, E.S.: Acquired coagulation factor deficiencies in leukemia. Cancer 26: 1097, 1970.

4) Goldstein, I.M., Wunschmann, B., Astrup, T., and Henderson, E.S.: Effects of bacterial endotoxin on the fibrinolytic activity of normal human leukocytes. Blood 37: 447, 1971.

ANNUAL REPORT

OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

The Experimental Therapeutics area functions primarily as three discrete but closely coordinated research units: 1) The Laboratory of Toxicology, whose primary responsibility is to investigate and describe the qualitative and quantitative toxicity of potential antineoplastic drugs using animal test systems and to conduct basic research on general principles of toxicology; 2) The Laboratory of Chemical Pharmacology, whose purpose is to study and elucidate the qualitative and quantitative aspects of the physiological disposition of antitumor agents in man, laboratory animals and in vitro predictive systems and to conduct basic research on general principles of pharmacology; 3) The Laboratory of Molecular Pharmacology, whose function it is to conduct basic research on the mechanism of action of antitumor agents at the molecular level and to understand how these drugs interact and how the biochemical and physiological consequences of such interactions are expressed. Summary reports of the individual laboratories highlight the research projects associated with their respective areas.

The Office of the Associate Scientific Director for Experimental Therapeutics, in addition to an over-all administrative responsibility for the three laboratories, conducts a research effort in the areas of (1) the physiological aspects of the blood-brain barrier and the pharmacologic behavior of substances (physiological and foreign) which traverse this barrier and (2) the biochemical pharmacology and toxicology of antitumor drugs in preclinical systems. Associated with the basic research being conducted in the former area, are efforts to devise a brain tumor model in monkeys and dogs which will permit the study and evaluation of antitumor drugs potentially active against brain neoplasms in man. Details of the research projects associated with the Office are found in the individual annual reports.

The successful integration of all the research activities associated with the Experimental Therapeutics area and the close cooperation of its scientific investigators and other personnel reflects the high calibre of leadership and scientific qualities of Dr. David P. Rall, who resigned as Associate Scientific Director effective April 13, 1971, to become the Director of the National Institute of Environmental Health Sciences. With the National Cancer Institute, Dr. Rall achieved scientific distinction in many areas associated with basic and clinical research. Notable was his pioneering effort in the development of the Chemotherapy program Linear Array for the pharmacological and toxicological evaluation of antitumor drugs destined for the clinic and his scientific research contributions which increased our understanding of drug action, especially in regard to the blood-brain barrier.

He also attracted and trained a number of outstanding laboratory and clinical investigators who have since distinguished themselves in the scientific community. The members of the Experimental Therapeutics area extend their fond wishes to "Dave" in his new role and look forward to his continued advice and counsel in our mutual scientific endeavours.

ANNUAL REPORT

OFFICE OF ASSOCIATE SCIENTIFIC DIRECTOR FOR EXPERIMENTAL THERAPEUTICS

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

Studies of the transport of various physiological and pharmacological substances between blood, brain, and cerebrospinal fluid (CSF) in normal, hydrocephalic, and plexectomized animals have been undertaken. Intravascular infusions and ventriculocisternal perfusions have been the methods generally used. Determinations of brain capillary and cellular permeability to a variety of physiological materials (inorganic ions, amino acids, and monosaccharides) have been made in normal animals. At present this work is being extended to plexectomized and hydrocephalic animals. These same parameters will be studied in brain tumors if an adequate model system can be developed. Efforts are underway to generate such a brain tumor model in monkeys and dogs. From work on normal and plexectomized monkeys, we have found that a significant portion of the CSF is formed by the brain parenchyma. The interactions of ions such as sodium and chloride and of drugs such as ouabain in the formation and flow of extrachoroidal CSF are being examined. It has been found that the rate of CSF production and the size of the brain extracellular space can be changed by osmotic imbalances between brain plasma and CSF and by the intraventricular infusion of various drugs. From work on elasmobranchs, we have successfully demonstrated that the CSF serves as a transient sink or drain for various materials which are produced by the brain or enter the brain from the blood at moderate rates. Our work with inositol has shown that this compound cannot be utilized for brain ventriculography as our earlier work had suggested.

Serial No. NCI-3500
1. Office of the Associate Scientific Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Penetration of Antitumor Drugs into the Central Nervous System

Previous Serial Number: Same

Principal Investigators: Joseph D. Fenstermacher and David P. Rall

Other Investigators: Ronald B. Blasberg, Daniel E. Weiner, Clifford S. Patlak, Walter Eckman, Mary Kathryn Hammock and Thomas Milhorat

Cooperating Units: Biometrics Branch, NIMH; Neurosurgery Service, NINDS

Man Years

Total:	6.5
Professional:	4.5
Other:	2

Project Description

Objectives:

To quantify the transport of various materials of physiological and pharmacological importance between blood, brain, and cerebrospinal fluid (CSF) and to study the effects of various pharmacological agents and pathological conditions upon the transport and metabolism of essential materials, e.g. amino acids, within the blood-brain-CSF system. It is the purpose of such studies to characterize the transport properties of the blood-brain-CSF system and to aid the development of chemical methods for obtaining more effective central nervous system (CNS) concentrations of antitumor drugs.

Methods Employed:

The methods of ventriculocisternal and ventricle-ventricle perfusion of various radioactive materials has been utilized in monkeys (normal and plexectomized) and dogs (normal and hydrocephalic). In addition studies of brain and CSF uptake and distribution of intravenously infused radioactively labeled physiological and pharmacological materials have been examined in dogs and monkeys.

Major Findings:

From our ventriculocisternal perfusion studies we have measured the size of the CNS extracellular space (ECS), the regional variation in this space within the brain, and the effects of tumor growth, hydrocephalus, plexectomy and drugs, e.g. ouabain, upon this space. From these same experiments we have shown with various radioactively labeled compounds, the adequacy of the ECS to serve as a pathway for the distribution of pharmacological agents and physiologically important materials from the blood and CSF to the brain tissue. With these methods we have been able to measure some of the kinetic parameters of ependymal permeability, cellular uptake, capillary permeability and cellular binding of various substances and changes in these parameters associated with the previously mentioned pathological conditions.

The rate of CSF secretion can be measured by determining the degree of dilution of ventriculocisternally perfused inert marker substances such as blue dextran, inulin, and albumin. It appears that the most accurate values for the production rate are obtained with albumin and blue dextran. The ^{14}C-inulin data yield a slight overestimation of this rate. Measurements of the production rates with ^{3}H-inulin data leads to sizeable overestimations (10-20%) of the secretion rate. It was also found that the osmolality of the perfusion fluid must be accurately matched to that of the endogenous CSF to obtain reliable production rate measurements. Using this same technique in plexectomized monkeys, we have determined that a significant amount of CSF is produced extrachoroidally.

Measurements of the diffusion coefficients of many organic materials in water and tissue and the effects of temperature, crystal formation, and some histological fixatives upon this process have been determined. Both freezing and fixing of tissue appear to stop diffusion of material within brain tissue.

We have continued to pursue studies in the comparative physiology and pharmacology of blood-brain-CSF transport with a particular emphasis upon the ontogeny and phylogeny of the blood-brain barrier. Marked differences in the transport of polar compounds between blood and brain and blood and CSF have been found for immature and mature elasmobranchs. The blood brain barrier appears to be much less developed in the immature forms of the same species. In addition the blood-brain-barrier in adult elasmobranchs found in warm waters ($22^{o}C$) seems to be more leaky than the blood-brain-barrier in adult elasmobranchs living in colder water ($12^{o}C$). Further studies of the distribution kinetics of polar materials in other body tissues, e.g. heart, liver, and skeletal muscle, have shown that the size of the extracellular space in these tissues is generally similar to those measured in mammalian species but that the rates of capillary and cellular uptake are slower in the elasmobranchs than in mammals. This work was done at the Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine; the Mote Marine Laboratory, Sarasota, Florida; and the Lerner Marine Laboratory, Bimini, The Bahamas.

An attempt to develop an improved system for performing brain scans was made using the biological compound inositol. Although the preliminary studies suggested that this material would give adequate scanning resolution to be used as a diagnostic technique, subsequent work indicates that inositol does not have the necessary physiological qualities to be used for this procedure.

The Brain Tumor Chemotherapy Study Group has been actively functioning both as a research forum and a collaborative neurosurgical group for the evaluation of chemotherapy in malignant brain tumors. Patient and data accrual is proceeding satisfactorily and new collaborating members are being integrated into the working protocol program. The first protocol study of the group, a controlled study of Mithramycin in glioblastoma with comparative analysis of survival time of postoperative patients, under the joint sponsorship of NCI and NINDS, has been completed. Mithramycin did not increase survival time. A new protocol using X-ray, surgery and BCNU alone and in combination is underway and progressing well.

Significance to Bio-medical Research and the Program of the Institute:

Tumors, both primary and metastatic, frequently develop in the central nervous system. Little information is available concerning the penetration of antitumor drugs into the CNS or into neoplastic disease within the brain. Basic research on this aspect of the pharmacology of antitumor drugs is necessary for the rational use of these agents. Furthermore, it becomes clear that the central nervous system acts in many ways as a typical cell and knowledge of the entry of drugs into the central nervous system parallels the entry of drugs into cells in general. The differences, however, between the central nervous system and other cells in general can and are being exploited to chemotherapeutic advantage.

Special transport systems appear to exist for moving various necessary material, e.g. amino acids, from blood to brain and CSF in normal animals and for removing various end products of metabolism, e.g. weak organic acid, from the brain and CSF. It is necessary to study and understand these mechanisms and quantitate them for normal brain and CSF. Similar studies of brain tumors and other pathological brain and CSF conditions should also be made in an attempt to examine the physiology, biochemistry, and metabolism of these diseases. The type of work performed in our laboratory on this subject is important and meaningful for an understanding of brain and meningeal tumors.

The Brain Tumor Chemotherapy Study Group functions as an initial effort in a controlled prospective study of the palliative effect of chemotherapeutic agents on malignant brain tumors, attempting to extend the methods of other collaborative chemotherapy programs in other diseases to the virtually virgin area of malignant neoplasms of the central nervous system.

Proposed Course:

1. Develop an adequate brain tumor model to use for physiological and pharmacological studies.

2. Continue the studies of the extracellular space and its function in normal, hydrocephalic and tumor animals. Proceed to further analyze the transport of various materials including drugs between blood, brain cells, and cerebrospinal fluid in normal, hydrocephalic and tumor animals.

3. Further attempts to elucidate some of the basic pharmacological factors determining both how much and how fast drugs get into the CNS and the spinal fluid from plasma. These include cerebral blood flow, structural similarity to various endogenous materials, etc.

4. Continued investigation of the comparative pharmacology of CNS and CSF. The technical ease with which the ventricular fluid, choroid plexus, and brain are obtained in elasmobranch make these species particularly valuable.

5. Continue to examine the mechanism and sites of CSF secretion and the effects of various drugs and pathological conditions on this.

Publications:

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2. Fenstermacher, J. D., Rall, D. P., Patlak, C. S., and Levin, V. A.: Ventriculocisternal perfusion as a technique for analysis of brain capillary permeability and extracellular transport. In Crone, C. and Lassen, N.A. (Eds.): Alfred Benzon Symposium II, Capillary Permeability. Copenhagen, Munksgaard, 1970, pp. 483-490.

3. Levin, V. A., Fenstermacher, J. D., and Patlak, C. S.: Sucrose and inulin space measurements of the cerebral cortex in four mammalian species. Amer. J. Physiol. 219: 1528-1533, 1970.

4. Curran, R. E., Mosher, M. B., Owens, E. S., and Fenstermacher, J. D.: Cerebrospinal fluid production rates determined by simultaneous albumin and inulin perfusion. Exp. Neurol. 29: 546-553, 1970.

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7. Fenstermacher, J. D.: Ventriculocisternal perfusion as a technique of studying transport and metabolism within the brain. In Marks, N. and Rodnight, R. (Eds.): Methods in Neurochemistry. New York, N.Y., Plenum Publishing Corp., in press.

Serial No. NCI-3516
1. Office of Assoc. Sci. Dir. for Exp. Ther.
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical, Pharmacologic, Toxicologic and Chemotherapeutic Aspects of Selected Antitumor Agents

Previous Serial Number: Same

Principal Investigator: J. A. R. Mead

Other Investigators: B. J. Abbott, N. H. Greenberg, A. Goldin, H. B. Wood, Jr. and J. Driscoll, NCI; W. R. Jondorf, George Washington University; A. C. Sartorelli and J. R. Bertino, Yale University; A. E. Auletta, C. T. Hardesty, L. C. Mishra, R. Tomchick and V. S. Waravdekar, Microbiological Associates, Inc.; Professor J. LeMen and M. M. Stenuit, Universite de Rheims, France

Cooperating Units: None

Man Years

Total:	3
Professional:	2
Other:	1

Project Description:

Objectives:

To investigate the biochemical pharmacology and biochemical toxicology of antitumor agents in experimental preclinical systems to better define the parameters which influence the chemotherapeutic activity of new or established agents.

Methods Employed:

Standard laboratory procedures were employed.

Major Findings:

The studies in this project can be considered under the following headings:

A. Folic Acid Antagonists
B. Homofolate Derivatives
C. 5-Hydroxypicolinaldehyde thiosemicarbazone

D. Ellipticine and its Derivatives
E. Sangivamycin
F. 1-Acetyl-2-picolinoylhydrazine
G. Emetine and Other Antiprotozoal Compounds as Antileukemic Agents
H. Studies on Resistance to Antileukemic Agents

A. Folic Acid Antagonists

As a result of the continuing activities in the contract with Stanford Research Institute several new analogs of methotrexate have been synthesized. These compounds include the following: 2',5'-dichloromethotrexate, 2',6'-dichloromethotrexate, 2',3'-dichloromethotrexate, 3',5'-dimethylmethotrexate, N_{10}-allylaminopterin, 3'-trifluoromethylmethotrexate, 3'-nitromethotrexate and m-glutamylmethotrexate. The synthesis of these structures was suggested by a retrospective analysis of structure-activity relationships in the antifolate area. The objective of this study is to define more closely the structural requirements needed for optimum antileukemic activity. The pharmacological basis for the differing toxicities and antileukemic effects of aminopterin, methotrexate and dichloromethotrexate remain uncertain but the additional information from studies with the compounds listed above should contribute much to our understanding of the biochemical pharmacology of this useful group of antitumor agents. The first studies indicate that in 3',5' disubstituted derivatives of methotrexate a bulky electronegative group is necessary for optimum activity as both 3',5'-dimethylmethotrexate and 3',5'-difluoromethotrexate have increased toxicity and lessened antileukemic activity in mice when compared with 3',5'-dichloromethotrexate. The comparisons are being conducted in vivo against advanced leukemia L1210 in mice as it was felt that use of early L1210 or in vitro inhibition of dihydrofolate reductase would not be sufficiently discriminatory to permit ranking the compounds.

B. Homofolate Derivatives

Further work on the reduced derivatives of homofolate has shown that it will probably be possible to obtain a useful antileukemic agent in this series which is stable enough to consider for further trial. This agent is 5-methyl-dl,L-tetrahydrohomofolate which on evaluation in vivo against leukemia L1210 and L1210/FR-8 was found to produce significant prolongation of survival time in both tumors whereas the parent compound does not have important activity against L1210. While the 5-methyl derivative has this very exciting activity the 5-formyl derivative which is analogous to citrovorum factor is also being evaluated as it may be even more stable than the 5-methyl compound.

The activity of the 5-methyl derivative against L1210 was strongly suggested a priori by the studies that have been conducted on the physiological disposition of tetrahydro and dihydrohomofolate. These studies have shown that the dihydrofolate reductase present in tissues is necessary for eliciting a maximal antileukemic effect. That this is the case was established by pretreating L1210/FR-8 leukemic mice with a low dose of methotrexate and following with either tetrahydrohomofolate or dihydrohomofolate. The antitumor effect of both derivatives was greatly reduced by this treatment schedule and

did not appear to be caused by increased toxicity. Further evidence for the involvement of dihydrofolate reductase in the biological activity of the reduced homofolates was obtained by isolating appreciable amounts of dihydrohomofolate from the livers of mice when treatment with either tetra- or dihydrohomofolate was preceded by methotrexate. In the absence of methotrexate both compounds can only be detected as the tetrahydro form. The significance of this is that tetrahydrohomofolate, the presumed antileukemic agent, has to be maintained in its reduced form by dihydrofolate reductase in vivo otherwise it will be oxidized to dihydrohomofolate which has no effect on thymidylate synthetase and which apparently has no antileukemic activity per se.

Other studies showed that the amount of dihydrohomofolate converted to tetrahydrohomofolate in L1210-FR8 infiltrated spleens was much greater than in L1210-infiltrated spleens. The most probable basis for the differential antileukemic effect seems to be related to the dihydrofolate reductase levels of the tumor. In view of this it is quite likely that unless a human tumor was found which contained high levels of the enzyme neither tetrahydro or dihydrohomofolate would have appreciable activity in man because of the relatively low levels of reductase in human tissues. This would be because sufficient dihydrohomofolate would not be reduced in the first place and the tetrahydrohomofolate would not be maintained in the fully-reduced form needed for antitumor activity. 5-methyltetrahydrohomofolate appears to lack these drawbacks in that it does not require dihydrofolate reductase for activity or decompose at a rate which would reduce its biological effect in vivo.

In related studies on the biochemical mechanism of action of homofolate it was found that the incorporation of orotic acid into DNA thymine was strongly inhibited by tetrahydrohomofolate although it has been reported by others that the incorporation of deoxyuridine into DNA thymine was not inhibited by the drug. This finding strongly supports the postulate that a major site of action of tetrahydrohomofolate is probably thymidylate synthetase although the lack of effect on deoxyuridine incorporation into DNA remains to be explained. Other biochemical studies have shown that the impact of single doses of tetrahydrohomofolate on incorporation of precursors into DNA is negligible whereas multiple doses exert an appreciable effect. It remains to be seen whether 5-methyltetrahydrohomofolate has any effects on precursor incorporation into DNA.

In further investigations of the effect of folate and homofolate on the dihydrofolate reductase activity in the kidneys of treated mice it was found by diafiltration that the inhibition observed in homogenates was due to the presence of large amounts of the free folates. Diafiltration removed the small molecular weight solutes while holding back the enzyme and as the concentration of folate in the retentate decreased the enzyme activity was restored. Since the precipitation of folates in the kidney is followed by a surge of DNA synthesis and mitosis it appears that mechanical effects rather than biochemical effects are responsible for the response. It had been hoped originally that this kidney response would be a model system of synchronized cells to study the impact of antitumor agents applied at various stages of the cell cycle.

C. 5-Hydroxypicolinaldehyde thiosemicarbazone

This drug which has significant antileukemic activity against leukemia L1210 in mice has been evaluated in man as an antitumor agent. It was found to have marked nauseating properties and this may severely curtail its usefulness as an antitumor agent. Physiological distribution studies in mouse and man reveal that the compound is rapidly and extensively transformed *in vivo*. In both species the major metabolite appears to be the glucuronide (40% of dose in mice and 70% in man). For this reason an attempt is being made to isolate the glucuronide in sufficient quantity to determine whether it has antitumor or nauseating properties. The glucuronide would presumably be on the 5-hydroxyl group and should not influence the thiosemicarbazide function which appears to be responsible for the antileukemic activity.

In mice the drug is rapidly excreted and in 24 hours 80% of the intraperitoneally administered dose is found in the urine and only about 25% of this is unchanged drug which appears soon after the drug is given. When the same dose is administered orally only 40% of the drug-derived radioactivity appeared in the urine in 24 hours and of this only 6-7% was as free drug and 9% as glucuronide. This lack of oral absorption may be a reflection of ionization or decomposition in the stomach but the lack of antileukemic effect by orally-administered drug supports the observation that it is not well-absorbed from the gastrointestinal tract.

In mice the disposition of the drug was followed after multiple-dose intraperitoneal administration on the q3h schedule which is the optimal treatment schedule for L1210. It was found that there was a greater persistence of unchanged drug in the tissues on this regimen, particularly in the spleen where 3% of the total activity after 96 hours could be attributed to unchanged compound. This observation may account to some extent for the considerable antileukemic activity of the drug on this optimal schedule.

In the clinical study it was found that there was an appreciable excretion of iron in the urine coincident with the administration of the drug. This probably reflects the agents ability to chelate the metal ion but it is not clear at present whether this is the basis for the antileukemic action *in vivo*.

D. Ellipticine and its Derivatives

Further studies of the disposition of ellipticine have been conducted in mice. Tissue levels were determined after oral administration in suspension and were found to be approximately equal to those obtained by intraperitoneal administration of a suspension. Subcutaneous administration of the drug in solution resulted in the lowest tissue levels and this correlated well with the poor antileukemic response seen with this route of administration. Lactate and hydrochloride salts of the drug exhibited similar patterns of distribution *in vivo*. Preliminary studies of the disposition of ellipticine-^{14}C showed that there was no evidence for any metabolites in any of the tissues examined. However, most of the radioactivity present in the urine was not present as ellipticine but as a metabolite.

9-methoxyellipticine has been used clinically in France as the lactate salt. A comparative study was conducted on the influence of routes and schedules of administration on the anti L1210 activity of ellipticine and its 9-methoxy derivative. 9-methoxyellipticine can also be determined in biological material according to the method worked out for ellipticine. The results of these studies showed that both compounds have similar toxicologic and antitumor effects and that their physiological disposition is very similar. The effects of the two compounds on the isolated dog heart is also similar. The recent report that ellipticine hydrochloride produces transient hemolysis has not yet been confirmed with 9-methoxyellipticine.

E. Sangivamycin

The distribution of tritiated sangivamycin has been followed in mouse tissues at time intervals from 30 minutes to 12 days after administration of a single dose. There studies have revealed that sangivamycin is extensively retained by the tissues mainly in the phosphorylated form. At times up to 12 days after administration the drug could still be detected in its free form and in the nucleotide pool of blood, liver, spleen, kidney, heart and brain. On day 12 levels between 1 and 5% of the maximum levels were present in all tissues except in the heart where the levels remained elevated. Radioactivity could also be detected in the RNA and DNA of all organs. Again at 12 days, levels remained elevated in the heart. The $t_{1/2}$ in mouse blood was quite prolonged, 72 hrs and in this respect sangivamycin resembles its congener tubercidin. In excretion studies the drug-derived radioactivity was found to be excreted slowly by the kidneys but only 40% of the drug equivalents could be accounted for in the urine in 12 days.

The persistence of drug-derived radioactivity in mouse tissues, especially in the heart, may correlate with reported cardiac toxicity seen in man and puppies. In any event it would appear that multiple treatment with this drug might be attended by toxic hazards. It is noteworthy that the optimal antileukemic schedule against leukemia L1210 is a single dose given early in the course of the disease. All other treatments are devoid of significant antileukemic activity and multiple treatments are significantly more toxic to the animals than the single dose.

F. 1-Acetyl-2-picolinoylhydrazine

Disposition studies of radiolabeled drug in the tissues and tumor of Walker 256-bearing rats have been conducted. Free drug was found to account for 10-30% of total radioactivity present in the tissues whereas in the 24 hr urine only 4-5% of the total radioactivity was present as free drug. This indicates that extensive metabolism of the drug occurs and two major metabolites and several minor ones have been demonstrated in the urine. Attempts are being made to characterize the major metabolites.

G. Emetine and Other Antiprotozoal Compounds

Emetine has been shown to have significant antileukemic activity against

L1210 when administered on an optimal schedule. Several other derivatives were also shown to have activity in the L1210 system. In view of this it was considered important to see whether the distribution of emetine hydrochloride after different schedules of administration could be related to the observed antileukemic effect in mice. After administration of a single intraperitoneal dose unchanged emetine can be detected in the livers, spleens, kidneys and hearts of treated animals within 15 minutes and can still be detected after 7 days. On a μg/g basis emetine was most concentrated in the spleen. After a 5 day treatment course in which the same total dose of drug is given a similar time course is observed for emetine tissue distribution as was seen after a single dose. Emetine concentration reaches the same levels in the livers and spleens following multiple dose administration as it does after a single injection but the levels in kidney and heart are significantly lower. Emetine was not detected in the brain after either treatment schedule.

In view of the antileukemic activity of emetine and its congeners other antiprotozoal agents are being examined for possible antileukemic activity. Two of these are niridazole and hetrazan, neither of which has had an extensive evaluation for antitumor activity.

H. Studies on Resistance to Antileukemic Agents

It has long been known that resistance to the folic acid antagonist, methotrexate, is often accompanied by an elevation in the levels of dihydrofolate reductase in the tumor cells. The mechanism underlying this response is not clear, however, a reasonable hypothesis would be that the accumulation of large quantities of enzyme-inhibitor complex within the tumor cell eventually results in a breakdown in the normal control mechanisms for synthesis of the enzyme and excessive amounts continue to be synthesized whether or not the drug is present. To investigate this aspect of resistance to methotrexate an investigation is underway to determine whether resistance, accompanied by increased levels of dihydrofolate reductase, develops when citrovorum factor is administered together with methotrexate. This treatment schedule would not inhibit the formation of the enzyme-inhibitor complex but it would nullify the biological effects of methotrexate. After 7 transplant generations no increase in reductase has occurred as a consequence of the combination treatment which may be indicative that the inhibition of the enzyme is not the trigger for the development of resistance *per se*.

Significance to Biomedical Research and the Program of the Institute

The acquistition of knowledge pertaining to the physiological distribution and metabolic transformations of antitumor agents is a very important objective of the drug development program of Chemotherapy, NCI. This project is directed towards the goal of acquiring this information and also to undertake more fundamental studies to elucidate the relationship, if any, of physiological distribution to chemotherapeutic activity and/or toxicity. This program is an integral part of the scientific endeavor under the aegis of the Pharmacology Committee of the Chemotherapy Program.

Proposed Course of Project

The project will continue to follow a course aimed towards the acquisition of relevant pharmacological, toxicological and biochemical data for input into the Linear Array for drug development. Concurrently, phenomena of a basic nature will be investigated inasmuch as they are related to the physiological effects, in animals, of experimental antitumor agents.

Publications:

Schrecker, A. W., Mead, J.A.R., Greenberg, N. H. and Goldin, A.: Dihydrofolate reductase activity of leukemia L1210 during development of methotrexate resistance. Biochem. Pharmacol. In Press.

Schrecker, A. W., Mead, J.A.R., Greenberg, N. H. and Goldin, A.: Dihydrofolate reductase activity and loss of resistance in an antifolate-resistant subline of leukemia L1210 after discontinuation of treatment. Chem.-Biol. Interactions 2: 229-234, 1970.

Tomchick, R. and Mead, J.A.R.: A method for the determination of 5-hydroxypyridine-2-carboxaldehyde thiosemicarbazone (NSC-107,392) in biological material. Biochem. Med. 4: 13-23, 1970.

Jondorf, W. R., Abbott, B. J., Greenberg, N. H. and Mead, J.A.R.: Increased lifespan of leukemic mice treated with drugs related to (-)-emetine. Chemotherapy 16: 109-129, 1971.

Mishra, L. C., Parmar, A. S. and Mead, J.A.R.: The effect of pretreatment with methotrexate on the reduction of dihydrohomofolic acid in mice. Biochem. Pharmacol. In Press.

Serial No. NCI-6109
1. Office of Associate Scientific
Director for Experimental Therapeutics
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971
*

Project Title: Tumor Growth Kinetics and Chemotherapy

Previous Serial Number: Same

Principal Investigator: Stanley E. Shackney

Other Investigators: Marc Straus

Cooperating Units: Dr. Lewis Lipkin, Pathology Section PRB, NINDS

Man Years

Total:	1.25
Professional:	1.25
Others:	0

Project Description:

Objectives:

1. To develop a model which will describe the growth of tumors in such a way that response to various chemotherapeutic agents can be simulated.

2. To apply the growth model to specific tumor systems.

3. To explore the pharmacodynamics of chemotherapeutic agents in specific tumor systems with the aid of the model.

4. To develop an approach to chemotherapy of tumors based on the expected interaction between tumor growth kinetics and drug pharmacodynamics.

Methods Employed:

Computer modeling techniques, *in vivo* and *in vitro* cell propogation techniques, radioautography with tritiated thymidine.

Major Findings:

a) Over the past year the computer model developed for tumor growth and chemotherapy of solid tumors has been expanded in scope, and has been developed into a general purpose simulation system for proliferating mammalian cell populations and their responses to various perturbations. This system may simulate normal or neoplastic cell populations, in hosts

ranging from mouse to man, in vitro or in vivo, with cross-culturing capabilities. Simulated experiments may include growth measurements, mitotic index, viable cell fraction measurements, stathmokinetic studies, tritiated thymidine studies and measurement of associated kinetic parameters, and finally, drug or radiation therapy using a variety of dosages, schedules, and concentration x time relations.

The model has been programmed in a question-answer conversational mode, so that users who are not familiar with computers may interact with it without difficulty. In addition, an on-line interactive graphic display program package is included for use with a CRT display when it is available.

To date, the properties of growth curves, damped oscillatory behavior in systems approaching equilibrium, and periodic phenomena in equilibrium systems, have been studied.

A more detailed account of the model and its findings has been outlined in a memorandum to Dr. Rall dated February 12, 1971. The first of a series of papers describing the model is currently being prepared for publication.

b) Work with the model has suggested a number of improvements in experimental methods for measuring kinetic parameters in mammalian cell systems. The image processing facility headed by Dr. Lewis Lipkin is admirably suited for the development and automation of these methods.

Over the past year a close working relationship was established with Dr. Lipkin and his group. Experience was gained in the operation of the computer operated miscroscope supervisory system, the scanning of cell images, and computer assisted cell sizing. There has been participation in systems design development, and responsibilities have been assumed for the development of an automated check list, automated documentation routine, and participation in the blood cell identification and grain counting project.

Laboratory Work:

1. Extensive data on growth rate and its variability in the Ca755 mouse tumor have been collected by Dr. Marc Straus.

2. Tissue samples for radioautography in this tumor have been collected at two different points in the growth curve, and are currently awaiting technical advances for further processing.

3. Attempts to obtain suspension from solid tumor tissue were abandoned when it was found that during the suspension procedure mitotic cells were lost. This suggested an experiment to determine the integrity of mitotic figures as a function of time following removal of tissue from the living host. Ca755 tumor, spleen, liver, and ilium were removed from mice at 5, 10, 20, 30, and 60 minutes after death of the host, for fixation. 0 time animals were perfused fixed. Data on mitotic indices in these tissues is

currently being processed.

Significance to Bio-medical Research and the Program of the Institute:

Hopefully, a better understanding of the growth characteristics of tumors, and the way in which growth affects response to chemotherapeutic agents, will lead to a rational, systematic approach to the chemotherapy of tumors, both experimentally and clinically, and provide the means for translating experimental data obtained in animals into clinically useful information.

Proposed Course:

a) The modeling of DNA synthesis rate characteristics and the development of a radioautographic simulation package should be completed over the next six months. A stathmokinetic drug simulation package for colchisine and vinblastine will follow. These two simulation packages will provide the necessary modeling tools for subsequent exploration of the kinetic mechanisms of commonly used chemotherapeutic agents.

b) Extensive collaboration with Dr. Lipkin and his group is anticipated, with the purpose of developing automated instrumentation for comprehensive kinetic characterization of mammalian cell systems experimentally.

c) The laboratory will serve as the experimental arm of the computer model. Experimental work will involve the kinetic characterization of a particular cell system *in vivo* and *in vitro*. The relations between the two will be determined. This system will be used to explore kinetic drug mechanisms, and test the model.

Publications:

1. Shackney, S. E.: A Computer Model For Tumor Growth and Chemotherapy, and Its Application to L1210 Leukemia Treated with Cytosine Arabinoside, (NSC-63878). Cancer Chemotherapy Reports: 54:399-429, part 1, No. 6, 1970.

2. Mendelsohn, M. L., Shackney, S. E.: The Growth Kinetics of Solid Tumors: Summary Report of Meeting. Cell Tissue Kinetics: 3:405-414, No. 4, 1970.

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This project was previously entitled Solid Tumor Growth Kinetics.

ANNUAL REPORT

LABORATORY OF CHEMICAL PHARMACOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 - June 30, 1971

The Laboratory of Chemical Pharmacology comprises three sections: (1) Biochemical Pharmacology Section; (2) Pharmacology and Experimental Therapeutics Section; and (3) Drug Metabolism Section. These sections function as a whole by conducting experimental investigations of new and established antitumor agents, covering many aspects of biochemical and comparative pharmacology, physiological disposition and experimental therapeutics. The Laboratory also collaborates with the Medicine Branch and Human Tumor Cell Biology Branch on clinical and laboratory research problems.

During the past year, Dr. David G. Johns, formerly Associate Professor of Pharmacology at Yale University, joined the Laboratory as Head of the newly established Drug Metabolism Section. Dr. Johns is internationally known for his classic work in folate and antifolate metabolism studies in man and laboratory animals. He is also an expert on the role of non-microsomal drug metabolizing enzymes in the catabolism of N-heterocyclic cancer chemotherapeutic agents. As the Section's Head, Dr. Johns is responsible for the use, application, and development of pharmacologic, physiologic, and biochemical techniques for the laboratory and clinical study of the metabolism, particularly by mammalian enzymes, and mechanism of action of cancer chemotherapeutic drugs and related compounds.

During the past year a number of basic research projects have been continued and new investigations initiated. Some of these studies are briefly discussed below.

The elucidation of the qualitative and quantitative aspects of the disposition of new and established antitumor agents in man and laboratory animals continues to be a major function of this Laboratory. The objective is to provide us with an improved understanding and broader knowledge of species differences in drug disposition to enable us to improve our abilities to predict pharmacologic and toxicologic properties of a given drug in man from data obtained in experimental animals. To achieve this objective, the Laboratory has adopted certain guidelines, defined in the Chemotherapy area Linear Array, for conducting physiological disposition studies. Relative to this program, the Laboratory continued during the past year to conduct preclinical and clinical pharmacologic studies on methotrexate (MTX), sodium camptothecin, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), and guanazole. Studies were initiated with 1-(2-chloroethyl)-3-(methylcyclohexyl) 1-nitrosourea (MeCCNU) and azathioprine.

Studies with MTX have primarily been concerned with the development of a pharmacokinetic model incorporating present data on this agent, to permit the design of more effective therapeutic doses and schedules for the clinic. Investigations have also been carried out on the role of intestinal bacteria in the metabolism of MTX and quantitative significance of this metabolic pathway in man. We are also continuing to study the kinetics of transport of MTX into sensitive and resistant leukemic blast cells in patients. Again, the objective here is to provide more complete qualitative and quantitative data on the basic and clinical pharmacology of this important and widely used antitumor agent.

Species differences in the plasma protein binding of sodium camptothecin were investigated during the past year and the data may provide some basis for explaining species differences in the toxicity of this novel alkaloid. Preliminary studies with the Lewis Lung-active agent MeCCNU indicate that this nitrosourea derivative behaves pharmacokinetically similarly to CCNU and BCNU. In rodents and dogs, oral administration is followed by rapid degradation of the drug and erratic prolonged absorption and excretion of molecular fragments primarily in the urine.

The evaluation of the usefulness of several induced primary and transplanted tumor systems as potential screens for clinically active compounds has continued during the past year. Particular attention was given to the evaluation of a number of antiviral agents, including Poly rI:poly rC, interferon, tilorone hydrochloride (interferon inducer) rifamycin SV, rifampicin (and derivatives) and amantadine derivatives. Also of particular interest was the evaluation of the in vivo and in vitro toxicity and antitumor activity of salts of the Group IIIa metals of aluminium, gallium, indium, and thallium. The spectrum of biological activity shown by the above antiviral agents and metal salts is described in the individual annual reports.

Because of the clinical importance of the potential embryotoxic and teratogenic effects of various anticancer and antiviral drugs, studies have also continued in this area. Various preparations of L-asparaginase were examined and results of these studies link the teratogenicity of this antitumor agent to a duel mechanism involving maternal depletion of L-asparagine and a direct action of the enzyme on embryonic tissue.

The availability of a large healthy breeding and experimental monkey colony consisting of 500 animals representing five species continues to provide the Chemotherapy program with a unique animal model system for the evaluation of the pharmacological, toxicological (including carcinogenic potential), and chemotherapeutic properties of anticancer, antiviral and antimicrobial agents. The induction of a reproducible tumor model (hepatoma) in a segment of these non-human primates has also provided a unique experimental system for conducting studies in experimental chemotherapy as well as on the fundamental aspects of hepatic tumor growth at the molecular level. These studies have been conducted during the past year and although total tumor regression was not seen upon treatment with methotrexate, camptothecin, poly I:poly C, or L-asparaginase, some evidence of growth suppression was noted grossly or by measurement of α-fetoprotein levels. Of particular significance during the

past year was the induction, in five of six owl monkeys inoculated with Herpesvirus saimiri (HVS) of malignant lymphoma with lymphocytic leukemia. This provides another subhuman primate tumor model system for studying lymphoma and leukemia and developing various diagnostic tests and chemotherapy treatment programs.

Studies have also continued to elucidate the mechanism of action of various anticancer agents and to explain the development of drug resistance by certain cancer cells. In collaboration with the Human Tumor Cell Biology Branch, these studies have primarily involved poly I:poly C, CCNU, and asparagine synthetase. The uptake of t-RNA into normal and leukemic cells has also been investigated. Briefly, the antitumor effect of poly I:poly C may be related to the combined action of interferon induction, inhibition of macromolecule synthesis and enhancement of immunological rejection of foreign antigens. In studies using sensitive and resistant leukemic L1210 cells, the therapeutic efficacy of CCNU is linked to prolongation of DNA synthesis. The control of asparagine synthesis has been linked to abnormal accumulation of amounts or of a specific asparagine-t-RNA.

In addition to our intramural research program, the laboratory is responsible for the administration and supervision of four contracts with outside research institutions. Three of these contracts are primarily concerned with the development of analytical-biochemical methodology and the study of the pharmacological disposition of antitumor drugs and their metabolites in man, laboratory animals, and in in vitro predictive systems. Approximately 12 agents are studied each year and reflect the interests of the LCP and Chemotherapy Program Area in developing antitumor drugs for the clinic. The remaining contract is concerned with the maintenance of a subhuman primate colony for the evaluation of potential carcinogens for man and the induction of primate solid tumors and leukemias which can be used as model systems to predict the usefulness of antitumor drugs in man.

Serial No. NCI-3502
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology and Drug Metabolism Sections
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Studies on Folic Acid Antagonists

Previous Serial Number: Same

Principal Investigators: David G. Johns, Daniel S. Zaharko, Donald M. Valerino, James A. Brown, and Vincent T. Oliverio

Other Investigators: Charles L. Vogel, Robert L. Dedrick, and Richard H. Adamson

Cooperating Units: Medicine Branch, NCI and Biomedical Engineering and Instrumentation Branch, DRS

Man Years:

Total:	5.0
Professional:	4.0
Other:	1.0

Project Description:

Objectives:

I. Development of a Mathematical Model for Describing the Pharmacokinetics of Methotrexate (MTX) in Laboratory Animals and Man

A pharmacokinetic model is merely a mathematical formulation concerning the known information about drug transport and drug-tissue interactions in vivo. If the information is sufficiently complete and accurate, then mathematical equations can be written describing the animal's handling of the drug. If the solutions of these equations under different initial conditions (species, dose, schedule) are predictive when checked against experimentally collected data, the model may be useful in the design of therapeutically effective doses and schedules. If the solutions are not predictive, insight into perhaps important lack of knowledge concerning the drug-tissue interactions may be gained.

II. Metabolism of Methotrexate by Intestinal Bacteria

Recent studies from this laboratory have shown the presence of at least

two MTX metabolites in urine and feces of mice and rats after the administration of tritium-labeled MTX. The conversion appears to be carried out by intestinal bacteria of these species during enterohepatic circulation of the drug. The objectives of the present study are to identify these metabolites and to determine their toxicity and biological activity as inhibitors of dihydrofolate reductase, relative to that of the parent compound, MTX. Studies will then be carried out to determine the quantitative importance of this possible metabolic pathway for MTX in man. The long-term objective of these studies is to provide more complete data on the basic and clinical pharmacology of this important antineoplastic agent.

III. Transport of Methotrexate and Metabolites Across Mammalian Cell Membranes

Studies are continuing to quantitatively determine the mechanism of the in vitro transport of MTX, its metabolites and analogues into sensitive L1210 leukemia cells and into sensitive and resistant human leukemic blast cells. Studies are also being conducted to determine the effect of commonly employed anti-inflammatory, antibiotic, and other antitumor drugs on MTX transport into these mammalian cells. Methods are being devised for separating human leukemic blast cells from other cellular elements in whole blood and/or bone marrow.

IV. Clinical Pharmacology of Folate Antagonists

While many aspects of the clinical pharmacology of MTX and other folate antagonists have been elucidated, a number of gaps still exist in our knowledge of the pharmacology of these agents. Among areas which still require study are the relative importance of the biliary route of excretion for MTX and for 3',5'-dichloromethotrexate (DCM) and the rate of metabolic inactivation of DCM by normal human liver tissue and by primary hepatocellular carcinoma tissue. Advantage is being taken of an on-going project being conducted at the Uganda Cancer Institute by Dr. C. L. Vogel, Medicine Branch, NCI, on the clinical evaluation of DCM and MTX in primary hepatocellular carcinoma, to carry out studies on these areas of MTX and DCM pharmacology.

Methods Employed:

Chromatographically pure MTX-3',5'-T was employed throughout. Drugs were administered by the intravenous, intraperitoneal, or intraduodenal routes. Standard surgical procedures and specimen collection techniques were employed throughout. In other experiments, mouse caecal contents were incubated with tritium-labeled MTX and the metabolites recovered. MTX metabolites were characterized by their chromatographic behavior in several systems and by their ultraviolet and infra-red absorption spectra. Large amounts of MTX metabolites were prepared by cleavage of MTX by bacterial carboxypeptidase.

Tentative identifications were confirmed by synthesis, followed by comparison of the properties of the synthetic compounds with those of the naturally occurring metabolites. The toxicity of the metabolites relative to that of the parent MTX was estimated. The activity of the metabolites as inhibitors of dihydrofolate reductase from mouse L1210 leukemia cells was determined relative to that of MTX. In patients with primary hepatomas receiving antifolate therapy at the Uganda Cancer Institute, frozen samples of plasma, urine, liver biopsy, and other biological materials are shipped to the Laboratory of Chemical Pharmacology, NCI for determination of drug levels and of DCM-oxidase enzyme activity by a direct spectrophotometric method. The methodology employed for short-term in vitro uptake and efflux studies of MTX in L1210 leukemic cells and human leukemic blast cells was adapted from the procedures of Goldman et al. (J. Biol. Chem. 243:5007, 1968). The human serum albumin density gradient procedure was used for enrichment of blast cell population from blood and bone marrow specimens containing mixed white blood cell types.

Major Findings:

I. Pharmacokinetic Modeling

The work over the past year, in attempts to make the model more accurate, have brought to light certain problems and unknowns which need to be investigated further if the total action of methotrexate in vivo is to be more fully understood.

A. Gastrointestinal Absorption:

MTX absorption rate appears to be governed by both Michaelis Menten kinetics (an active saturable process) and by diffusion. The latter process takes on considerable importance at high doses where much of the drug appears intraluminally as a result of active biliary secretion. Preliminary studies have indicated that certain antibiotics (neomycin, gentamicin) affect this process. It is logical to expect that the drug itself (MTX) may change the absorption process since it has cellular effects in the intestine. Subsequent doses may behave differently than the first dose as a result of these effects. These problems are being looked into.

B. Tissue Transport In Vivo:

This model has indicated that the marrow is relatively unimportant in determining the overall plasma history of MTX. However, we do know that this tissue is critical in manifestation of toxicity to this drug. We have therefore done some preliminary studies to investigate drug transport in vivo into marrow following single pulse injections. We have preliminary data in rats to suggest that a time dependent transport is involved. Although several investigators have characterized an active transport system in vitro, we know of no verification of its importance in vivo.

C. Model Applications:

To test the thoroughness of our knowledge about the pharmacological behavior of MTX we have attempted to predict from one basic model what should happen to drug disposition, in several species. The nature of the model allows these predictions if adjustments are made for known species differences in drug-tissue interactions and known species differences in basic physiological parameters (sizes, blood flow rates, etc.). Through the mammalian species: mouse, rat, dog, and man we have been fairly successful, in a preliminary way, of determining what factors play important roles in determining concentrations of the drug at various organ sites with time.

We have investigated the pharmacokinetic behavior of this drug in the tissues of some primitive fish (elasmobranchs). The similarity of the physico-chemical tissue-drug interactions are striking. If physiological differences are also taken into account, we believe we may use the mouse model to account for the drug's distribution in species as wide apart as primitive fish and mammals. Studies are currently in progress to achieve this comparison and hence gain further basic knowledge in drug-tissue interactions.

II. Bacterial Metabolism

The major MTX metabolite in feces has been identified as 4-amino-4-deoxy-N^{10}-methylpteroic acid (APA). This compound is also the major MTX metabolite observed on incubation of MTX with mouse caecal contents *in vitro*. APA was found to be a moderately effective inhibitor of dihydrofolate reductase (DHRF) from mouse L1210 leukemia cells ($K_i = 2 \times 10^{-8}$ M), and a substrate for rabbit liveraldehyde oxidase ($K_m = 2.2 \times 10^{-4}$ M); the product of oxidation of APA by the latter enzyme was 4-amino-4-deoxy-7-hydroxy-N^{10}-methylpteroic acid. In *in vivo* studies, APA has been recovered from the feces of CDF_1 mice in the 6-hr period after the intraperitoneal administration of tritium-labeled MTX; trace amounts of APA could also be detected in urine collected over this time-period. The major MTX metabolite in the urine of the mouse, however, was a compound other than APA; from its chromatographic behavior, the major urinary metabolite of MTX appears to be a free pteridine, but whether the pteridine metabolite arises directly from cleavage of MTX or indirectly *via* the cleavage of APA, is not yet established. These studies provide direct support for the hypothesis of Zaharko *et al.* (Science 166:887, 1969) that in the mouse, and possibly in other mammalian species, MTX is subject to extensive metabolism by intestinal bacteria.

III. Transport Studies

The *in vitro* uptake of MTX by sensitive L1210 cells is unidirectional until high affinity intracellular binding sites (presumably to DHRF) are saturated.

The rate of uptake and total intracellular concentration of MTX at equilibrium are both proportional to the extracellular MTX concentration. After equilibration of preloaded cells in MTX-free buffer, rapid efflux is observed to a concentration of approximately 8-12 mμM MTX/gm drug cell weight.

The MTX metabolite, APA, is taken up in a linear and temperature independent manner by MTX sensitive L1210 cells which is compatible with a passive diffusion process. In human leukemic blast cells from patients clinically resistant to MTX, uptake of MTX at 2 μM concentrations was not observed, but a low linear uptake of APA was apparent.

Glucose, cephalothin (Keflin), heparin, and aspirin were found to have a net inhibitory effect on the in vitro uptake of MTX by L1210 cells. Michaelis-Menten studies have shown the uptake inhibition due to cephalothin to be competitive in nature, possibly the result of a common membrane carrier molecule for transport of weak organic acids into the cell. No effect of cephalothin or the other compounds on MTX efflux has been observed.

IV. Clinical Pharmacology

Rates of metabolism of DCM by liver biopsy material from 3 normal subjects and 15 patients with hepatocellular carcinoma have been assayed. Assessment of these results is in progress.

In an ancillary study, the rate of MTX secretion in human milk was determined. The patient was an East African nursing mother who was receiving MTX for choriocarcinoma. Milk levels of MTX after an oral dose of 15 mg/m^2 were found to reach a maximum level of 5×10^{-9} M, while plasma levels reached 1.8×10^{-7} M and urine levels reached 6.8×10^{-5} M. The ratio of milk:plasma:urine MTX levels was thus 1:36:13,600, i.e., milk is a minor route of excretion of MTX and it would appear that these patients could continue to nurse without danger to the infant from MTX toxicity. This question is of some practical importance with reference to the treatment of patients with antifolates under African conditions. Studies on a second choriocarcinoma patient receiving both MTX and actinomycin D are being carried out.

Significance to Bio-medical Research and the Program of the Institute:

Among the active antitumor folic acid antagonists, MTX is the most widely used, and is clinically effective in the treatment of acute lymphocytic leukemia, choriocarcinoma, Burkitt's lymphoma, primary hepatocellular carcinoma, and epidermoid cancer of the head and neck. Because of the great clinical importance of MTX, a complete knowledge of its pharmacology is of practical value. With this established agent it is envisioned that additional information on its distribution and metabolism in a variety of

species, and a further understanding of its uptake characteristics into malignant cells alone or in the presence of other drugs, will lead to its improved use and the design and use of newer more effective drugs.

Proposed Course:

1. Further studies will be conducted to make the mathematical pharmacokinetic model more accurate for MTX. This will involve the determination of the effects of antibiotics and multiple MTX doses on the absorption of MTX and further studies to characterize and quantitate the transport process into bone marrow and other tissues in vivo.

2. To identify the major urinary metabolite of MTX in experimental species (rat and mouse), followed by determination of its biological properties by the same methods as those described above for the major fecal metabolite of MTX.

3. Examination of human urine and feces after the administration of tritium-labeled MTX for the presence of MTX metabolites utilizing the same methods as those described above for MTX metabolites in the mouse.

4. If MTX metabolites are found in man, the effect of antibiotic administration on the levels of such metabolites and on the toxicity of MTX in man will be determined.

5. To determine what differences in MTX transport (if any) divide resistant from sensitive human leukemic cells.

6. To continue study of drug-drug interaction of MTX in L1210 cell system and to extend similar investigations to in vitro study of human leukemic cells and ultimately to in vivo studies in animals and humans.

7. Over the longer term, the course of clinical pharmacologic studies will be determined by the clinical results obtained in the treatment of primary hepatomas with DCM. If is hoped that sufficient data will be accumulated to test the hypothesis of a possible selective advantage of DCM over MTX in the treatment of these tumors. Ancillary clinical pharmacological studies on biliary and mammary gland secretion of DCM and MTX will also be carried out as clinical material becomes available; these studies are of value since some of this data is not presently available, but will be required for the complete development of a pharmacokinetic model of MTX and DCM distribution in man.

Publications:

1. Zaharko, D. S. and Oliverio, V. T.: Reinvestigation of methotrexate metabolism in rodents. Biochem. Pharmacol. 19: 2923-2925, 1970.

2. Zaharko, D. S., Dedrick, R. L., Bischoff, K. B., Longstreth, J. A., and Oliverio, V. T.: Methotrexate tissue distribution; prediction by a mathematical model. J. Nat. Cancer Inst., in press.

3. Oliverio, V. T. and Zaharko, D. S.: Tissue distribution of folate antagonists. Ann. N. Y. Acad. Sci., in press.

4. Johns, D. G. and Valerino, D. M.: Metabolism of folate antagonists. Ann. N. Y. Acad. Sci., in press.

5. Valerino, D. M., Johns, D. G., Zaharko, D. S., and Oliverio, V. T.: Studies of the metabolism of methotrexate by intestinal flora. I. Identification and biological properties of the metabolite 4-amino-4-deoxy-N^{10}-methylpteroic acid. Biochem. Pharmacol., in press.

Serial No. NCI-3505
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Richard H. Adamson and Vincent T. Oliverio

Other Investigators: None

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total:	1.0
Professional:	0.5
Others:	0.5

Project Description:

Objectives:

The susceptibility of newborn and adult animals to chemical carcinogens and, particularly, drugs of interest in cancer chemotherapy and antiviral agents and those to which the human population may be exposed is of vital necessity and of particular relevance to the program of NCI. The characterizations and treatment of such tumors is of both theoretical and practical importance. The specific objectives are:

To determine the carcinogenic activity of chemicals to which the human population may be exposed, particularly drugs of interest in cancer chemotherapy, such as procarbazine hydrochloride, guanazole, poly I:poly C, tilorone and derivatives, and polethylene diamine.

To characterize and transplant carcinogen-induced tumors as new tools for experimental cancer chemotherapy.

To compare the susceptibility of newborn vs. adult animals to chemical carcinogens.

Methods Employed:

Usual biologic, pharmacologic, and histopathologic techniques.

Major Findings:

Guanazole did not produce tumors when tested in CDF_1 mice. The drug was tested both as a single dose and in multiple injections and mice were sacrificed six months later without evidence of tumor.

Procarbazine hydrochloride, oral, or intraperitoneally (i.p.) induced pulmonary tumors in 70-100% of CDF_1 mice with a latent period of 16-25 weeks and leukemia in 40-90% of mice, varying with dose and route of administration. Similar results were obtained in non-inbred albino (Swiss) mice.

In rats procarbazine (oral or i.p.) induced mammary tumors in 89-95% female and 62% male Osborne-Mendel rats, but only one leukemia and 5 lung tumors occurred in 98 rats. In Fischer rats the mammary tumor incidence after procarbazine treatment with 50% in females but 37% of the rats had sarcomas of the kidney, 15% had squamous carcinomas of the skin, and 4% had sarcomas of the endometreal stroma.

Forty-five Rhesus, Cynomolgus, and African Green monkeys received procarbazine hydrochloride for periods up to 6 years. Two of these monkeys developed myelogenous leukemia and two others died with hyperplastic bone marrow (but not leukemia). Seventeen other monkeys died while under treatment with procarbazine and usually showed a depression of bone marrow and lymphoid tissue. Other lessions of interest (occurring more than twice) include ulceration of esophagus (3) and hyperplasia in the urogenital tract (3).

Significance to Bio-medical Research and the Program of the Institute:

Several drugs used as anticancer agents are known to be carcinogens. Few, however, have exhibited such strong carcinogenic activity as procarbazine, and none elicit such a wide spectrum of tumors in mice and rats. With the clinical use of procarbazine, it is essential to carefully delineate its carcinogenic potential, not only in rodents, but also in primates.

Guanazole is chemically related to the "triazole" that is used on cranberries which produced thyroid tumors in rodents. The possibility that guanazole may have a similar action was investigated. The possible carcinogenic activity of other antitumor agents (poly I:poly C) and other synthetic ribonucleic acids is not only of clinical importance, but theoretically, they may be of importance to help understand the malignant process.

Nitrosamines and nitroso compounds are possible carcinogenic materials in our environment. It is important to evaluate their potential hazard, both in the young and in the mature population.

Proposed Course:

New antiviral and anticancer agents of clinical interest will be evaluated as potential carcinogens. Various nitroso compounds that may occur naturally will also be tested for carcinogenic activity. Tumors that occur from various carcinogens will be evaluated for their applicability to programs for antitumor testing.

Publications:

1. Adamson, R. H., Fabro, S., Homan, E. R., O'Gara, R. W., and Zendzian, R. P.: Pharmacology of polyriboinosinic:polyribocytidylic acid a new antiviral and antitumor agent. In Hobby, G. L. (Ed.): Antimicrobial Agents and Chemotherapy - 1969. Bethesda, Md., American Society for Microbiology, 1970, pp. 148-152.

Serial No. NCI-3506
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Pharmacology of Alkylating Agents and Other Antitumor Drugs

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Michael M. Hart

Other Investigators: Sergio Fabro, Department of Pharmacology, The George Washington University

Man Years:

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description:

Objectives:

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

The possible embryotoxic effects of anticancer agents and antiviral agents that may be used clinically or even more generally are being investigated.

Methods Employed:

Usual pharmacologic, biochemical, immunologic, and histopathologic techniques.

Major Findings:

Poly rI:poly rC was found to be active in a mouse reticulum cell sarcoma (A-RCS) and in the Walker 256 survival system giving a ILS of 100%. Poly rI: poly rC had minimal, but definite, activity against leukemia L1210 and plasma cell tumor YPC-1, but it had no activity against several other leukemias, including P388 and K1964.

DDD Studies

o,p'-DDD has been shown to affect different species in different ways. In the dog, this drug (1) inhibits ACTH-induced adrenal steroid production, (2) prevents steroid metabolism by the liver, and (3) produces adrenal cortical atrophy. In the rat o,p'-DDT (1) does not affect adrenal steroidogenesis, (2) stimulates steroid degradation by the liver, and (3) has no effect on the size of the adrenal cortex. In Rhesus monkeys prolonged administration (i.p.) of 60 mg/kg o,p'-DDD in corn oil daily for 15 weeks (1) inhibits ACTH-induced adrenal steroid production, (2) induces hepatic steroid catabolism, and (3) causes atrophy of the adrenal cortex. From the limited data obtained in man, it appears that the monkey may be an even better model than the dog for studying the toxicity and antitumor activity of o,p'-DDD.

A cell suspension preparation from dog adrenal cortex has been developed. These cells respond to exogenous ACTH by synthesizing corticosteroids. Preliminary experiments using the different geometric isomers of DDD reveal that all 3(o,p'-p,p'- and m,p'-) inhibit ACTH-induced steroidogenesis. p,p'-DDT has no effect on steroid production. It has been known that both o,p'- and m,p'-DDD possess this action in vivo in dogs. It has not been established that p,p'-DDD also has this effect.

DDT Studies

p,p'-DDT (50 mg/kg/day) treatment of pregnant New Zealand rabbits early in their pregnancy (days 7, 8, and 9) produced (1) an increased incidence of premature births, (2) an increased number of fetal resorptions, and (3) a marked decrease in fetal weight on day 26 and 28 of pregnancy. The insecticide is not teratogenic at this dose. Treatment of does later in pregnancy (days 21, 22, and 23) had no effect on date of delivery, incidence of resorptions or fetal weight. Pesticide analysis of tissues of mother and fetus revealed that p,p'-DDT and its metabolites (p,p'-DDT, p,p'-DDE, and DDMU) are concentrated in both the fetus and in organs of the mother which regulate fetal environment.

Interaction of KI and Cyclophosphamide

It has been reported that potassium iodide may potentiate the antitumor activity of cyclophosphamide in the treatment of Burkitts lymphoma. The effect of potassium iodide on the acute toxicity and antitumor activity of cyclophosphamide in mice was investigated. Administration of potassium iodide (p.o., s.c., or i.p.) increased the lethality of cyclophosphamide over a 30-day observation period. Potassium iodide had no effect on the antitumor efficacy of cyclophosphamide against leukemia L1210 in vivo or in tissue culture.

Antitumor Activity of Group IIIa Metals

The in vivo and in vitro toxicity and antitumor activity of salts of the Group IIIa metals of aluminium, gallium, indium, and thallium were determined. Using the LD_{50} as a measure, the decreasing order of toxicity of compounds tested in both CDF_1 mice and Sprague-Dawley rats was $TlCl_3 \geq In(NO_3)_3 > Ga(NO_3)_3 > Al(NO_3)_3$. Utilizing i.p. doses $\leq$ the LD_{10}, the decreasing order of effectiveness against the Walker 256 carcinosarcoma both in vivo (ascitic) and in tissue culture was $Tl^{+3} > Al^{+3} > Ga^{+3} > In^{+3}$. Similar non-toxic doses of these salts were ineffective against leukemia L1210 both in vivo (ascitic) and in tissue culture. Experiments in which the route of Walker 256 tumor inoculation and drug administration were varied revealed that Ga^{+3} and, to a lesser extent, In^{+3} inhibited tumor growth when injected at a site differing from that of tumor inoculation. Both Tl and Al which were very effective in tissue culture and when injected i.p. into animals carrying the ascitic tumor were not effective when transport to the tumor on the blood was required. When these salts were tested against 8 solid tumors transplanted subcutaneously in rodents, $Ga(NO_3)_3$ was the most effective giving better than 90% tumor growth inhibition of 6 tumors. Both $Al(NO_3)_3$ and $In(NO_3)_3$ had some growth inhibitory actions against these tumors $TlCl_3$ had little or no activity against these tumors. None of the Group IIIa salts showed antitumor activity against various ascitic transplantable rodent leukemias, including L1210.

Embryotoxic Effect of L-Asparaginase

The embryotoxic activity of E. coli, crystalline E. coli, and Erwinia carotovora L-asparaginase has been evaluated. In the New Zealand White rabbit all preparations increased the number of resorption sites and determined the appearance of viable fetuses with congenital malformations. Chromosomal analyses of L-asparaginase treated mothers and fetuses showed normal karyotypes. In addition, L-asparaginase in vitro decreased the mitotic index of rabbit white blood cell cultures without altering the karyotype. E. coli L-asparaginase was without embryotoxic effects in the Sprague-Dawley rat.

Following the administration of an embryotoxic dose of L-asparaginase to rabbits, the enzyme persists in the plasma for more than 48 hours and crosses the placental barrier of the 8-day pregnant rabbit. These findings suggest that a duel mechanism involving (1) maternal depletion of L-asparagine and (2) a direct action of L-asparaginase on embryonic tissue, is responsible for the teratogenic effect of this enzyme.

Antitumor Activity of Antiviral Agents

The antitumor activity of various antiviral agents is being examined in both viral and non-viral tumors. Among the compounds being tested are interferon, interferon inducers, rifamycin SV, rifampicin, and derivatives, and amantadine derivatives.

Rifamycin SV is the most cytotoxic of all these compounds against in vitro L1210, Novikoff tumor, Walker 256, and human cell lines. Both rifamycin SV and rifampicin were active against ascitic Walker 256, but inactive against leukemia L1210 and P388 in vivo. Tilorone hydrochloride, a synthetic small molecular-weight inducer of interferon, had antitumor activity against Walker carcinosarcoma 256 and the reticulum cell sarcoma A-RCS in rodents. Tilorone hydrochloride was inactive when tested in several other rodent tumors including leukemias L1210 and P388. Tilorone hydrochloride also had cytotoxic activity in vitro against Walker carcinosarcoma 256, leukemia L5178Y, and the Novikoff hepatoma. In addition, the antitumor activity of tilorone hydrochloride and poly I: poly C in 3 rodent tumors was compared. Tilorone was inactive in the leukemia L1210 system, whereas poly I:poly C had minimal, but definite, activity against this tumor. Tilorone and poly I:poly C had comparable activity against reticulum cell sarcoma A-RCS, but tilorone was more active against the Walker carcinosarcoma 256 than poly I:poly C.

Significance to Bio-medical Research and the Program of the Institute:

Careful evaluation of the effect of antitumor compounds in induced tumor systems should be made until sufficient data is available to judge their usefulness in screening compounds for ultimate clinical trial. o,p'-DDD is used in the treatment of adrenocortical carcinoma and Cushings Syndrome. Because of the tissue and chemical specificity of this compound, it is important to delineate its pharmacology in full with the hope that this information will lead to the development of less toxic agents for the treatment of these conditions. Further study of this type of compound may also prove useful in treating other steroid related cancers (ovary, testis, and placental). The possible embryotoxic and/or teratogenic effects of the various anticancer and antiviral drugs is of clinical importance and also may help shed light on nutritional requirements of fetus and on possible mechanisms of contraception. A rational approach to the design, selection and testing of new anticancer agents is of utmost importance and supplements the screening approach of CCNSC. Knowledge of the metabolic fate of various anticancer agents, their plasma $t_{\frac{1}{2}}$, their route of disposition, and their interaction with other drugs will lead to better therapeutic treatment.

Proposed Course:

1. Use of induced tumors (primary and transplanted) for chemotherapy will be continued.

2. Studies of the embryotoxic effects of newer anticancer agents will continue.

3. A rational approach to the design, selection, and testing of potential anticancer agents will continue.

4. Studies of the metabolic fate of ^{14}C-cyclophosphamide in patients and of ^{14}C-guanazole in animals and man will continue.

5. Studies on the mechanism of the antitumor action of tilorone will begin and the antitumor activity of various interferon inducers and interferon *per se* will be compared.

6. We are further examining the possibility that potassium iodide may potentiate the antitumor activity of cyclophosphamide by studying the effect of this salt on the plasma half-life of cyclophosphamide in primates.

7. Further studies are being conducted to determine if the cell suspension system will be of predictive value in evaluating the potency and efficacy of drugs proposed for the treatment of adrenal cortical cancer.

Publications:

1. Adamson, R. H., Fabro, S., Hahn, M. A., Creech, C. E., and Whang-Peng, J: Evaluation of the embryotoxic activity of L-asparaginase. Arch. Int. Pharmacodyn. 186: 310-320, 1970.

2. Adamson, R. H.: Antitumor activity of two antiviral drugs - rifampicin and tilorone. Lancet p. 398, 1970.

3. Adamson, R. H.: Antitumor activity of tilorone hydrochloride against some rodent tumors: Preliminary report. J. Nat. Cancer Inst. 46: 431-434, 1971.

Serial No. NCI-3509
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Carcinogenesis in Primates

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Roger W. O'Gara (deceased Jan. 1971), and Dharam V. Ablashi

Other Investigators: Clyde W. Dawe, Jacqueline Whang-Peng, Robert Gallo, and Dan Dalgard

Cooperating Units: Laboratory of Pathology, Viral Leukemia and Lymphoma Branch, Human Tumor Cell Biology Branch, and Medicine Branch, NCI; Hazleton Laboratories, Inc., Falls Church, Va., and Bionetics Research Laboratories, Kensington, Md.

Man Years:

Total:	3.0
Professional:	2.0
Others:	1.0

Project Description:

Objectives:

This project is designed to obtain comparative data on the response of primates and rodents to known carcinogens and to materials suspected to be carcinogenic in man. In addition, the induced primate tumors will hopefully be useful as model tumors systems for man. Specifically the objectives are:

To study the effect of compounds, generally of potential carcinogenic activity in newborn primates and to determine if the newborn primate, like the rodent, will be highly susceptible to carcinogenesis.

To obtain data on which to base a comparison of the response of monkeys and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and primates in predicting for man.

To evaluate known and suspected oncogenic virus in subhuman primates and to utilize these tumors for chemotherapy with antiviral and antitumor agents.

To use normal and tumor-bearing primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

Methods Employed:

Usual pharmacologic, biochemical, clinical, and anatomical pathologic techniques and cytological and immunologic techniques. In addition, breeding nursing care of infant monkeys and special dietary regimens. The breeding record and health status of the breeding colony is similar to that reported for previous years. In addition, this year we have obtained owl monkeys (Aotus trivirgatus) and are attempting to establish a breeding colony of this species.

Major Findings:

General Information:

Previous annual reports have contained detailed information concerning the management and maintenance procedures employed in the breeding colony and information concerning the methods utilized in rearing of the newborns. These procedures have not been significantly altered and are, therefore, not included in this report.

The present colony consisting of 500 animals is comprised of five species: Macaca mulatta, Macaca irus, Cercopithecus aethiops, Galago crassicaudatus panganiensis, and Aotus trivirgatus. Of these, 365 are experimental; the remaining 135 are adult breeders which supply the newborns for experimental study.

All animals are housed in an isolated facility which contains only animals committed to this study. Most animals with the exception of the breeding colony are housed in individual cages. As newborns are produced by the breeding colony, they are delivered to the nursery area which is staffed on a 24-hour basis. Compound administration is generally initiated immediately.

Various physical and chemical parameters are monitored in each animal to evaluate the general health status and any evidence of tumorigenesis. Surgical biopsies of tumor specimens are obtained when indicated and submitted to the Laboratory of Pathology for histopathologic evaluation. All animals which die or are sacrificed are carefully necropsied and tissues examined microscopically.

No major disease problems have been encountered during this period. An usually high incidence of abortion and stillbirths were encountered during a one-month period early in the year. The condition was self-limiting and the causative factors were not determined.

During the past year, 16 of the breeding females were removed from the breeding colony because of poor reproductive histories and were used for physiological disposition studies of various anticancer agents. Sixteen replacement animals were purchased from Hazleton Laboratories' breeding stock colony. The only other animals brought in from outside sources were seven newborns which were donated to the program by Hazleton Laboratories.

Compounds of Particular Interest:

N-Nitrosodiethylamine (DENA)

This compound has continued to demonstrate potent carcinogenic properties. In general administration by the intraperitoneal route continues to produce a very high incidence (approximately 100%) of liver tumors which metastasize readily to the lungs and lymphatic tissues. The induction time is inversely affected by the level of administration and the frequency of administration, but generally ranges from 13 to 20 months. The shortest induction time has been eight months.

To date, liver tumors have been induced in 75 animals utilizing DENA. Forty-six of these have resulted following intraperitoneal injections generally given every 14 days at a maximum level of 40 mg/kg. The oral administration of DENA at the same level, but given five times weekly resulted in a lower incidence and longer induction time. A total of 29 hepatic cell carcinomas were induced by this method.

Generally, gross evidence of tumor is preceded by the development of a positive α-fetoprotein test. The time interval, however, is variable and as yet we are unable to draw conclusions concerning the factors affecting the occurrence of these two events.

Attempts at chemotherapeutic control of tumors have been attempted. Methotrexate, camptothecin, poly I:poly C, and L-asparaginase therapy have been given to a small number of animals. Total regressions were not affected, but some evidence of growth suppression by both gross measurements, α-fetoprotein levels, and histopathology was seen.

1-Nitrosopiperidine (PIP)

Two additional animals have developed liver tumor, bringing the total number of animals with tumors to three during this report period. In light of the chemical similarities between this compound and DENA, it seems reasonable to suspect that the intraperitoneal administration of PIP may result in a higher incidence and shorter induction time. A group of 10 animals presently about two years of age is on such a regimen.

N-Isopropyl-α-(2-methylhydrazino)-p-toluamide (Procarbazine)

Two monkeys on treatment with procarbazine have developed myelogenous leukemia and were characterized by their rapid course to an early death.

The first leukemia, which arose in a 17-month old Rhesus female (M. mulatta), was suspected for three months before death, but a definite diagnosis was made (by bone marrow biopsy) only a few weeks before death. During this time the peripheral WBC rose dramatically from 26,000 to 352,000, and immature white blood cells appeared in great numbers in the circulating blood. The second leukemia was even more rapid, and leukemia was not suspected until hemorrhagic nodules appeared in the skin and axilla a scant 36 hours before death. The second leukemia appeared to be more "acute" than the first one since, in addition to its more rapid clinical course many more immature white cells and fewer platelets were present in the peripheral blood. The ages of the two monkeys (17 and 67 months) corresponds roughtly to an age of 4 years and 16 years in the human since in our experience an infant Rhesus monkey matures about three times as fast as a human infant.

The young age of the monkeys, the rarity of myeloid leukemia in subhuman primates, and the fact that they were receiving a chemical known to induce leukemia in mice, suggest strongly that the leukemias were induced. Approximately 50 other monkeys have received the same chemical for up to six years without developing leukemia. However, 2 of 15 monkeys that died while receiving this compound had hyperplastic bone marrow, but not leukemia. Periodic blood counts and smears of monkeys on this compound have usually revealed a depressed white blood count rather than an elevated one. Observation of the remaining monkeys receiving this compound will supply valuable information on the latent period and probable incidence of this induced leukemia in primates. This information, in turn, will enable us to detect leukemia early enough to treat with one of the many antileukemic drugs which are continuously being developed.

Attempts to transmit these leukemias by intracerebral inoculation in newborn Rhesus monkeys (M. mulatta) have so far failed, although this method has been successful for transplanting monkey hepatoma after growth in tissue culture. No viruses have been detected in either of the leukemias by electron microscopic examination of tissue, by animal inoculation or tissue culture, although studies are not yet completed on the second leukemia.

3-Methylcholanthrene (MCA) and Benzo[α]pyrene (BP)

Various polycyclic hydrocarbons that produce malignant tumors in rodents have failed to produce tumors under similar experimental conditions in Rhesus, Cynomolgus, and Squirrel monkeys. We administered MCA and dibenzo-[α, i]pyrene orally and by injection into Rhesus and Cynomolgus monkeys. Single or multiple subcutaneous or intradermal injections induced keratoacanthomas, papillomas, and giant cell granulomas, but no sarcomas or carcinomas. These animals were observed 4-8 years.

In contrast, we induced tumors by MCA or BP in tree shrews and galagos after a single injection of the carcinogen. The tumors produced by the carcinogens in these prosimian primates were fibrosarcomas. Therefore, it seems possible that the prosimians, which include tree shrews, lemurs, lorises, and tarsiers, may be more closely related to the rodent than in the higher primates in their reactions to polycyclic hydrocarbon carcinogens.

Herpesvirus saimiri (HVS)

Five of six owl monkeys inoculated with HVS propagated in owl and African green monkey kidney cell cultures developed malignant lymphoma with lymphocytic leukemia. The virus was recovered from these monkeys and identified as HVS. The disease had a course of 55 to 70 days and was characterized by generalized lymphadenopathy and lymphocytic leukemia with circulating actively motile lymphocytes. The lesions were characterized by the obliteration of normal lymphoid tissue architecture by neoplastic lymphocytes and by neoplastic lymphocytic invasion of almost all organs. No virus was detected in the tissues by electron microscopy, however, upon cultivation HVS recovered.

Significance to Bio-medical Research and the Program of the Institute:

The susceptibility of non-human primates to known rodent carcinogens and to possible carcinogens in man is of theoretical, practical, and economic importance. In addition, studies of the carcinogenic potential of antitumor agents (e.g., procarbazine) will indicate whether these compounds should or should not be used clinically in patients without neoplastic disease for organ transplantation and in treating arthritis and other diffuse collagen disorders. The susceptibility of the newborn monkey vs. the adult monkey to carcinogens and other foreign compounds is information that is needed for the clinician and for judging the safety of various food additives, supplements, and other compounds to which man is exposed. In addition, tumors induced in non-human primates are likely to be more useful than the rodent tumor system now used to predict the usefulness of anticancer agents for man. In particular, the owl monkey may serve as a useful model for lymphoma and leukemia. Potential diagnostic tests for precancerous and malignant tissue may also be discovered by use of non-human primate tumors; few such tests now are useful for man.

Proposed Course:

Treatment with present carcinogens will be continued in the monkeys, and it is hoped that an observation period of at least ten years will give some basis for predicting which compounds are likely to be carcinogenic in man.

More owl monkeys will be inoculated with HVS and when disease is apparent treatment with known antileukemic and antiviral agents will be undertaken. In addition, an owl monkey breeding colony is being developed.

A continued effort will be made to assess the carcinogenic potential of procarbazine and to utilize these tumors for chemotherapy.

Treatment of monkeys with liver tumors will be continued with various chemotherapeutic agents.

Various monkeys will be utilized for comparative disposition studies and toxicological evaluation of anticancer, antiviral, and antimicrobial agents.

Studies will continue on the correlation of the production of α-fetoprotein in monkeys with the time of appearance of tumors and with histopathologic changes in the liver and in the tumors.

Studies of the methylase activities and the t-RNA profiles of fetal, normal, and hepatoma will be continued.

Studies of the metabolism of the various classes of carcinogens in various species will be continued. Such studies, it is hoped, will give more insight into susceptibility or refractoriness of primates compared to rodents.

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Serial No. NCI-3512
1. Lab. of Chemical Pharmacology
2. Biochemical Pharmacology Sec.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Biochemical Pharmacology of Newer Antitumor Agents and Model Compounds

Previous Serial Number: Same

Principal Investigators: Anthony M. Guarino and Vincent T. Oliverio

Other Investigators: Richard H. Adamson, Vincent T. DeVita, Jeffery A. Gottlieb Jerome B. Block, Ayub K. Ommaya, and Reginald L. Reagan

Cooperating Units: Medicine Branch, Baltimore Cancer Research Center, and Human Tumor Cell Biology Branch, NCI; and Surgical Neurology Branch, National Institute of Neurological Diseases and Stroke

Man Years:

Total:	5.0
Professional:	3.0
Other:	2.0

Project Description:

Objectives:

To study the biochemistry and pharmacology of camptothecin, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU), and L-asparaginase. To examine the metabolism and biliary excretion of certain antitumor agents and model compounds in rats and other species in order to better understand the mechansms of drug metabolism and biliary transport.

Methods Employed:

A. Camptothecin, sodium:

Standard assay techniques previously reported from this laboratory were used for the quantitative determination of this alkaloid in various physiological fluids and tissues in patients, animals, and the plasma ultrafiltrates from a variety of species. To determine plasma binding of camptothecin sodium to the plasma of these species, a new method employing ultrafiltration through a conical membrane filtration system was employed.

B. CCNU and MeCCNU:

Cyclohexyl-^{14}C-CCNU was employed in clinical pharmacologic studies in two patients with malignant glioblastomas. Following oral administration of the drug, blood, cerebrospinal fluid (CSF), and urine samples were collected at certain time intervals. Biopsy samples of brain and tumor tissue were also obtained. All samples were assayed for intact drug, decomposition products, and total radioactivity using thin-layer chromatography, combustion, and liquid scintillation counting techniques. Preclinical pharmacologic studies were initiated in rodents and dogs with MeCCNU labeled with ^{14}C in the ethylene moiety. The methodology for analyzing physiological fluids and tissues for parent compound and total radioactivity (parent plus decomposition products) was similar to the techniques employed in studies with CCNU.

C. L-asparaginase:

Standard biochemical methods were used in determining the levels of various drug metabolizing enzymes in control and phenobarbital-treated rats.

D. Model Metabolism and Biliary Transport Studies:

Standard methods for separation and isolation of microsomes and various sub-microsomal fractions were employed. The methods used for determining rates of drug metabolism in these microsomal preparations were as described previously in publications from this laboratory.

Major Findings:

A. Camptothecin, sodium:

1. Since it had been reported earlier that biliary excretion is an important mode of deposition of camptothecin, it was felt that another aspect of transport, i.e., the binding of the drug to plasma proteins, should be investigated. Accordingly, we studied the plasma binding of camptothecin sodium to a variety of species and the results are summarized below (mean % bound for 2-4 determinations):

Species	% Binding
Human	98.6
Monkey	95.8
Guinea Pig	93.7
Lobster	93.3
Rat	93.0
Harbor Seal	73.3
Dog	68.0
Mouse	54.8

Species	% Binding
Horse	52.1
Lamb	49.7
Pig	49.5
Cow	37.3
Chicken	33.2

2. Sixteen patients with various advanced solid tumors received single intravenous injections of 0.5-10.0 mg/kg at intervals of 2-4 weeks. Partial remissions (>50% mass decrease) were noted in five patients, and evidence of objective response was noted in six others. Responses occurred predominantly in patients with advanced and often refractory gastrointestinal carcinoma. Pharmacologic studies revealed that a high percent of the drug is bound to plasma proteins after intravenous administration, with drug detectable in the plasma for up to six days after injection. About 17% of unmetabolized drug was found in the first 48-hr urine collection. As yet no urinary metabolites, which are fluorescent, have been found in the urine of patients receiving this compound.

B. CCNU and MeCCNU:

1. Oral administration of 1.5 mg/m^2 and 2.0 mg/m^2 of ^{14}C-cyclohexyl CCNU to a female patient and male patient respectively resulted in rapid absorption and prolonged ^{14}C plasma levels. Peak plasma levels in both patients were achieved approximately 4 hours after drug dosage and persisted at about the same level through 8 hours. CSF and brain tumor tissue taken during the initial 8 hours contained about 25% and 50% respectively of concurrent ^{14}C plasma levels. In the female patient approximately 70% of the total administered radioactivity was excreted in the urine during the first 24 hours. In the male patient, urinary excretion of ^{14}C was considerably reduced, where only 23% of the dose was recovered. This was probably partially due to a loss of a urine sample. Radioactivity in plasma, CSF and urine samples was primarily lipid insoluble and did not correspond to CCNU.

   These results confirm earlier clinical pharmacologic data with CCNU and further indicate the selective localization of certain degradation products of the parent compound in brain tumor tissue.

2. The plasma disappearance of MeCCNU (as intact drug) was determined in mice after a single intraperitoneal injection. The half-life during the first 30 minutes of exponential fall was in the order of 6-8 minutes. This was followed by a slower disappearance phase. The results are similar to the plasma disappearance pattern observed with CCNU.

The distribution and excretion of MeCCNU was studied in mice each given a single intraperitoneal (i.p.) or oral (p.o.) dose of 36 mg/kg of the drug labeled with ^{14}C in the ethylene portion of the molecule. In mice given the drug i.p., excretion was primarily by the kidney where about 70% of the radioactive dose was recovered in the urine as degradation products after 24 hours. Approximately 8% of the radioactivity appeared in the expired air as $^{14}CO_2$. The remainder of the radioactive dose resided in the carcass (7-8%), intestines with contents (5-6%), liver (2-3%), and kidney (1%). Results following a single oral dose in mice were similar to the results obtained after parenteral administration. Less than 1% of the dose was recovered in fecal material after i.p. or oral MeCCNU after 24 hours.

The plasma disappearance, CSF levels, and excretion of radioactivity was measured in two dogs given a single intravenous injection of 15 mg/kg ethylene-^{14}C-MeCCNU. In both dogs, urinary recovery of ^{14}C was about 25% of the administered radioactive dose after 24 hours. Plasma ^{14}C levels, although steadily disappearing following injection, were measurably significant during the first 24 hours and were paralleled by equivalent or slightly higher CSF radioactivity up to 4 hours following drug administration. Liver, kidney, and spleen contained the highest levels of ^{14}C per gram of wet tissue amongst all tissues examined. In two dogs given a single oral 15 mg/kg of MeCCNU, absorption of radioactivity was rapid but erratic and steadily increased to peak in one dog at 5 hours, and in the other at 7 hours. Recovery of ^{14}C in the urine at 24 hours was about 95% and 43%, respectively. Analysis of bile, taken from the gall bladder at 24 hours revealed that about 2-4% of the dose resided in this physiological fluid. Liver, kidney, and spleen were highest in radioactivity in terms of tissue specific activity.

C. L-asparaginase:

The treatment of male albino rats with sufficient L-asparaginase to cause decreases in plasma albumin and gamma globulin, did not significantly alter the basal activities of aniline hydroxylase, ethylmorphine N-demethylase, p-nitrobenzoic acid nitroreductase.

D. Model Metabolism and Biliary Transport Studies:

1. Transport:

a. Biliary Excretion of Glururonides:

Because some compounds which are carcinogenic are excreted into the bile as glucuronides, e.g., N-hydroxy-2-acetylaminofluorene, we are studying the metabolism of a variety of glucuronide compounds and their parents in both ordinary Wistar rats and in the Gunn strain of rats which have a congenital deficiency in the ability to synthesize certain glucuronides.

So far, for two compounds studied, probenecid and phenolphthalein, it appears that the Gunn rat can synthesize the glucuronides of both of these compounds about 70% as well as the Wistar rat; there appears, however, to be a deficiency in the transport system.

b. Comparative Pharmacology of Biliary Excretion of Foreign Compounds

Because of the hypothesis that the biliary excretion of foreign compounds may have developed before renal excretion of such compounds, we studied a variety of acidic, basic, and neutral drugs in the spiny dogfish (Squalus acanthias), the following compounds were administered to dogfish and the plasma and bile of these animals was analyzed for the presence of the drug and its known metabolites: Probenecid, phenol red, camptothecin sodium, indocyanine green, penicillin G, DDT, cytoxan, methotrexate, and procaine amide ethobromide. For all of the compounds studied the 24-hr bile/plasma ratios were greater than unity. It was concluded that although biliary excretion by the dogfish seems to be a slower process than occurs in most mammalian species, this animal does utilize biliary excretion as a mode of excretion of foreign compounds.

c. Fate and Distribution of DDT in the Lobster

We were interested in studying the fate and distribution of DDT for the following reasons: First, because of its high lipid solubility it is a good model drug with which to compare the distribution of lipid soluble antineoplastic drugs. Second, because of the carcinogenic potential of DDT, we are interested in the distribution of this pesticide in any sources which reach man. After administration of ^{14}C-DDT to lobsters via injection into the paracardial sinus, we found that 7 days after this treatment, 97% of the administered dose appeared in the liver of the lobster. Other studies at earlier times showed that there was some accumulation in lobster gonads during the first 48 hrs after treatment. We chose to work with the lobster because this marine animal stores virtually all of its body fat in one organ, the liver. Thus by analyzing the lobster liver we could predict the potential importance of storage of a potential neoplastic agent in the adipose tissue of man.

2. Conditions Altering the Metabolism of Drugs

We are continuing our studies of a variety of physiologic-pathologic states which are known to alter drug metabolism. The condition of cachexia is often associated in patients with carcinoma and we have used total starvation of rats as a model of this emaciating condition. It was found that in male rats starved for 72 hrs, there is a significant increase in cytochrome P-450 content and the rates of metabolism of ethylmorphine and aniline.

It was further found that if the 72-hr starvation period was followed by a 72-hr re-feeding period that many of the parameters of drug metabolism activity had not yet returned to control levels. It was also observed that the effect of starvation on the kinetics of drug oxidation can vary markedly according to the sex of the rat employed and the particular drug substrate investigated.

a. Species and Strain Differences

In an effort to further our knowledge of the differences in responses which can be predicted from humans, we have extended our studies on the drug metabolizing activity of microsomes and submicrosomal fractions from the following species: guinea pig, monkey, and rabbit. We have also compared the drug metabolizing activities of microsomes isolated from the Wistar and Gunn strains of rats.

In the subfractionation of microsomes the procedure of Bergstrand and Dallner, Analytical Biochemistry 29:351, 1969, was used. In this subfractionation procedure a new type of centrifuge loader number 40.2 is employed. Electron microscopy standard methodology was employed to verify the completeness of the subfractionation using this new procedure.

1. Microsomal Subfractionation Studies: Work in two other species, the rat and the rabbit, indicated that microsomal drug metabolism activities and cytochrome P-450 were generally concentrated in the smooth membranes rather than the rough membranes. However, for one enzyme studied UDT-glucuronyl transferase activity for some substrates was shown to be concentrated periopherentially in the rough membrane fraction of rabbit liver. In the present work we have investigated three other species, the guinea pig, the mouse, and the monkey, to see if the submicrosomal distribution of several enzyme activities compared with the findings of earlier species studied.

   The validity of the microsomal fractionation technique was appraised by electron microscopy and biochemical analyses. In the guinea pig and monkey there was a three-fold enrichment of RNA in the rough as compared with the smooth fraction. In the mouse, however, the RNA level is only twice as great in the rough fractionation and the RNA values differ greatly from both those of the guinea pig and the monkey. Other investigators have reported that there is a three to six-fold enrichment in cholesterol in smooth membranes and, indeed, we were able to verify this finding in our studies in the mouse liver; however, this did not occur in the monkey or the guinea pig.

In the guinea pig and the monkey the smooth to rough ratios for ethylmorphine demethylase, l-benzphetamine demethylase, and aniline hydroxylase activities were all greater than one, indicating their concentration in the smooth membrane. The UDT-glucuronyl transferase activity was greater in rough membranes in all three species. Hence, the submicrosomal distribution for this enzyme is markedly different from that in the guinea pig and monkey than the other enzymes studied.

2. Microsomal Cytochrome P-450 Reductase Activities:

Since a good correlation was observed between drug metabolism and the rate of reduction of cytochrome P-450 by NADPH under a number of diverse conditions, it was suggested that P-450 reductase activity might be the rate limiting step in the microsomal oxidation of drugs. In the present study we have extended the number of experimental conditions where drug metabolism is known to be altered. When the rate of P-450 reductase was reported in terms of the concentration of cytochrome P-450, there were significant differences observed when controls were compared with phenobarbital-treated animals when controls were compared with 3-methylcholanthrene-treated animals, and when control animals were compared with starved. There were no significant differences when males were compared with females or when adults were compared with immature animals. Therefore, the data indicates that changes in drug oxidation are frequently, but not invariably, accompanied by an equivalent difference in the rate of cytochrome P-450 reductase activity.

The studies in the Gunn rat were conducted because this animal provides an interesting model of what may be happening in a patient with liver function impaired and resulting in hyperbilirubinemia. For example, in the Wistar animal the bilirubin levels are less than 1 mg/100 ml whereas in the Gunn rat the levels of bilirubin are more than 12 mg/100 ml. In comparing the Wistar and the Gunn jaundiced rats, it was found that the amounts of microsomal protein, cytochrome P-450, and cytochrome C, were not significantly different in the microsomes; not only was the rate of formation of phenolphthalein glucuronide diminished, but also we found an impairment in the metabolism of ethylmorphine. The latter finding is important because this pathway is not the same as glucuronidating enzymes and has not been reported to be deficient in Gunn rats.

Significance to Bio-medical Research and the Program of the Institute:

In the studies with L-asparaginase an experimental design was used which may be of general applicability in the early assessment of interactions that can occur with drug combinations to be used in cancer chemotherapy.

For example, we were able to detect a statistical and biochemical interaction which occurred when phenobarbital was co-administered with L-asparaginase. In the biliary excretion studies involving the Gunn rat it appears that in this strain of rat there are differences not only in the rate at which it can synthesize a particular metabolite, but there are more significant differences in the rate at which it can transport this metabolite into the bile. The studies in the lobster provide a method for the early assessment of the possible role of adipose tissue in sequestering a lipid soluble antineoplastic drug. By studying different species of animals as well as imposing some pathologic condition such as starvation, we should be able to make better extrapolations of animal data to humans.

Proposed Course

1. Further studies of the pharmacologic disposition of camptothecin will be conducted, especially along the lines to try to determine the metabolic fate of this compound since to date we have only been able to account for about 50% of the drug.

2. Clinical pharmacologic studies with the Baltimore Unit have been completed.

3. Preclinical pharmacologic studies will be continued with MeCCNU and clinical pharmacologic studies will be initiated during Phase I trial of the drug in solid tumor patients.

4. Investigators in this section will begin to use the combination gas chromatographic-mass spectroscopic techniques to study the metabolism of antineoplastic agents such as camptothecin and CCNU.

5. Studies will be initiated on the clinical pharmacology of a parenteral form of procarbazine hydrochloride.

6. Biliary transport studies will be continued on various model drugs commonly used in patient therapy will be tested to see if they alter the rate of biliary excretion of various potential antineoplastic agents.

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Serial No. NCI-6108
1. Lab. of Chemical Pharmacology
2. Pharmacology and Experimental Therapeutics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Mechanisms of Action and Mechanisms of Resistance to Anticancer Agents

Previous Serial Number: Same

Principal Investigators: Richard H. Adamson and Robert G. Gallo

Other Investigators: None

Cooperating Units: Human Tumor Cell Biology Branch and Medicine Branch, NCI and National Institute of Allergy and Infectious Diseases

Man Years:

Total:	1.5
Professional:	1.0
Others:	0.5

Project Description:

Objectives:

The knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To design new anticancer agents utilizing knowledge obtained so that a higher cell kill can be obtained and the problem of resistance can be circumvented.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis.

In addition, transfer RNA is prepared by standard techniques and iso-accepting species of t-RNA are isolated by use of a Freon reverse phase partition column.

Major Findings:

This year we have been concerned with the mechanism of action of poly I: poly C, CCNU, and the control of asparagine synthetase. In addition, we have investigated the uptake of t-RNA by normal and leukemic cells.

Studies on the antitumor activity of poly I:poly C lead us to conclude that its effect is due to a superimposition of 3 effects; first, to a direct action of interferon which it induces; second, direct and selective chemotherapeutic action on the tumor by inhibition of macromolecule synthesis; and third, on enhancement of immunological rejection of foreign antigens.

Uptake of t-RNA (Escherichia coli) was demonstrated in the murine leukemia, L1210 and in a human lymphoblast (NC-37) cell line. In both cell lines, uptake of t-RNA was rapid, reaching a maximum within 45 sec, and was linear with concentration up to about 50 μg/ml. This uptake of exogenous t-RNA apparently was not due to altered membrane permeability or impaired cell viability, nor to ribonuclease degradation of the macromolecule. Furthermore, about 20% of the t-RNA taken up by the cells remains functional and apparently intact. This was demonstrated by: (a) acylation with E. coli aminoacyl-t-RNA synthetases; (b) methylation with leukemic cell t-RNA-methylases, and (c) demonstrating ^{14}C-labeled 4S RNA in the cytoplasmic fraction of the leukemic cells after the addition of E. coli [^{14}C]t-RNA. The results demonstrate that t-RNA can enter mammalian cells and suggest that an energy independent, carrier-mediated, mechanism may be operative.

Our observations open the possibility of testing the biological effects of various foreign t-RNAs in mammalian cells. It has been shown that the addition of aminoacyl-t-RNAs to chick blastodiscs largely reversed the inhibition of hemoglobin formation produced by actinomycin or 8-azaguanine. Previous findings led to the suggestion that t-RNA may exert regulatory functions in cell differentiation, that changes in t-RNA may lead to neoplastic transformation, and that specific aminoacyl-t-RNAs may be involved in transcriptional regulation. It is conceivable that experiments based on a nonphysiological input of foreign t-RNA might help clarify any regulatory role of t-RNA. The control of asparagine may be due to abnormal accumulation of amounts or of a specific asparagine-t-RNA (Asn-t-RNA). Various Asn-t-RNA species were characterized by column chromatography from asparaginase resistant L1210 and 6C3HED/R and asparaginase sensitive L5178Y and 6C3HED mouse leukemic cells, and from normal mouse liver and spleen. Three species of Asn-t-RNA were identified in L1210 and 6C3HED mouse leukemias and in normal tissues. However, in tumors sensitive to L-asparaginase there was a relative increase in peak 2 Asn-t-RNA and less of peak 1 as compared to cells resistant to L-asparaginase. In addition to these differences, the sensitive leukemia L5178Y had a fourth species of Asn-t-RNA.

Therefore, it is hypothesized that Asn-t-RNA peak 2 and possibly peak 4 may be the repressor for asparagine synthetase.

Studies on 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and its degradation products on the duration of DNA synthesis in nitrosourea sensitive and resistant leukemia L1210 were performed and indicated that the characteristic prolongation of DNA synthesis noted with the nitrosourea derivative 1,3-bis(2-chloroethyl)-1-nitrosourea was also produced by CCNU of the postulated decomposition products of CCNU, only cyclohexyl-isocyanate produced a similar effect, and survival studies in sensitive and resistant strains of leukemia L1210 tend to link the prolongation of DNA synthesis by these drugs with their therapeutic efficacy, but improved survival in sensitive tumor strains was not produced by the degradation product cyclohexylisocyanate.

Significance to Bio-medical Research and the Program of the Institute:

Knowledge of the mechanism of action of anticancer agents will lead to the design of newer drugs, hopefully, with greater cell kill and less toxicity. Knowledge of the mechanism of resistance to various anticancer agents will lead to measures that minimize development of resistance or will overcome this resistance. In addition, there is the possibility that resistant cells can be converted into sensitive cells.

Proposed Course:

The mechanism of antitumor activity of several newer anticancer agents, including camptothecin, guanazole, tilorone, gallium, and other metals will be investigated. In addition, we will test t-RNA peak 4 to see if this will result in repression of asparagine synthetase and hence sensitivity to therapy with L-asparaginase to derepressed leukemic cells (L1210).

Publications:

1. Herrera, F., Adamson, R. H., and Gallo, R. C.: Uptake of transfer ribonucleic acid by normal and leukemic cells. Proc. Nat. Acad. Sci. 67: 1943-1950, 1970.

2. Levy, H. B., Asofsky, R., Riley, F., Garapin, A., Cantor, H., and Adamson, R.: The mechanism of the antitumor action of poly I:poly C. Ann. N. Y. Acad. Sci. 173: 640-648, 1970.

3. Gallo, R. C., Longmore, J. L., and Adamson, R. H.: Asparaginyl-t-RNA and resistance of murine leukemias to L-asparaginase. Nature 227: 1134-1136, 1970.

Serial No. NCI-6111 (new)
1. Lab. of Chemical Pharmacology
2. Drug Metabolism Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Routes of Catabolism of Azathioprine

Previous Serial Number: None

Principal Investigator: David G. Johns

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Other:	0

Project Description:

Objectives:

Azathioprine (6-(1-methyl-4-nitroimidazo-5-yl)-thiopurine; Imuran) an imidazolyl derivative of 6-mercaptopurine (6-MP), is active both as a cancer chemotherapeutic and an immunosuppressive agent, and is in general clinical use for the latter purpose. Knowledge of the metabolism of this drug is still incomplete; it is known from animal studies, however, that it undergoes oxidation both by aldehyde oxidase and by xanthine oxidase. The objective of this study is to determine in several species, including man, the relative quantitative importance of the routes of metabolism of azathioprine. The effect of agents which inhibit the enzymes responsible for azathioprine catabolism on the in vivo therapeutic effectiveness and the toxicity of azathioprine will be examined. The possibility will be examined of enhancing the effectiveness of this drug as an antineoplastic and immunosuppressive agent by inhibiting its catabolism and thus by diverting more of the drug into the anabolic pathways which convert azathioprine and/or 6-thiopurine to the active nucleotide form.

Methods Employed:

Products of azathioprine catabolism have been identified by ultraviolet and infra-red absorption spectroscopy, by thin-layer and paper chromatography, and by carbon-hydrogen-nitrogen combustion analysis.

Aldehyde oxidase, xanthine oxidase, and other enzymes and enzyme-systems involved in azathioprine catabolism have been purified by differential centrifugation, ammonium sulfate fractionation, gel filtration, and other standard enzyme purification methods. In work with crude tissue homogenates and with particulate suspensions, incubation with substrate and cofactors will be followed by separation and isolation of oxidation products. The effects of known inhibitors of aldehyde oxidase and of xanthine oxidase on the catabolism of azathioprine will be studied *in vitro* and *in vivo*.

Major Findings:

The rate of oxidation of azathioprine to 8-hydroxyazathioprine by aldehyde oxidase from rabbit liver and from human liver (necropsy specimens) has been determined. Azathioprine was found to be a rapidly oxidized substrate for the enzyme from both species, its V_{max} being *ca.* 3-fold that for substrates such as N-methylnicotinamide and 6-methylthiopurine. The reaction product was isolated and purified, and found to be identical in its chromatographic and spectrophotometric properties with synthetic 8-hydroxyazathioprine. *In vitro*, the aldehyde oxidase-catalyzed oxidation of azathioprine was found to be significantly inhibited by menadione, estradiol-17β and Triton X-100. These studies are still in their initial stages and are continuing.

Significance to Bio-medical Research and the Program of the Institute:

Azathioprine is active both as an antitumor agent and as an immunosuppressive agent. It is in general clinical use for the latter purpose; its antitumor activity, however, is comparable to that of 6-mercaptopurine and 6-thioguanine. It is highly probable that inhibition of the catabolism of azathioprine *in vivo* will result in an increase in its pharmacological activity; whether its therapeutic index will also be increased remains to be determined, and is one of the objects of the present study.

Proposed Course:

On completion of the present studies on the oxidation of azathioprine by drug metabolizing enzymes *in vitro*, the effect of the more potent inhibitors of azathioprine catabolism will be assessed *in vivo*. Species utilized initially will be mice, rats, and monkeys. Urines will be collected and urinary oxypurines and thiopurines determined by standard chromatographic methods. If difficulty is encountered in detection of metabolites, 8-^{14}C-labeled azathioprine will be prepared and utilized in these studies as an aid to product identification. In mice, the therapeutic efficacy and toxicity of azathioprine will be examined in the presence and absence of inhibitors of azathioprine catabolism; the test systems used will be the S-180 mouse tumor and the suppression of antibodies to sheep red cells, as determined by hemagglutinin titer. In the other species cited, attention will be directed primarily to the effect of inhibitors on the pattern of oxypurine and thiopurine metabolites excreted.

If warranted from the human enzyme *in vitro* data and the animal *in vivo* data, studies of azathioprine catabolism in man will be carried out in cooperation with the Medicine Branch, National Cancer Institute. The long-term objective of these studies is to devise methods to increase the clinical usefulness of azathioprine as an antineoplastic and immunosuppressive agent in man.

ANNUAL REPORT

LABORATORY OF MOLECULAR PHARMACOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

The objectives of this laboratory are to study the mechanisms of drug effects related to nucleic acid structure and function, and ultimately to help relate these effects to chemotherapeutic properties.

The major efforts during the past year have been along three lines:

1) Effects on purified DNA. During the past year we have concentrated on anthramycin. This compound is of special interest because its structure, high specificity, and manner of binding are unique among DNA-binding agents. It is hoped that these properties will permit the use of anthramycin as a probe of DNA structure. When this is applied to DNA in chromatin or in conjunction with other DNA-binding agents, it may lead to a mapping of DNA with respect to different classes of sites. Viscosity and sedimentation studies indicated that anthramycin stiffens the DNA molecule, but does not intercalate between base-pairs. Studies of the reactivity with defined polynucleotides indicated that anthramycin binds only to deoxyribo-polymers that contain G. Studies with intact L1210 cells showed that DNA is the major or sole intracellular site of tight binding. Subsequent studies will focus on the relationship between the sites for anthramycin and the sites for the binding of other drugs or of nuclear proteins.

2) Effects on RNA metabolism in L1210 cells. The objective here is to investigate the detailed effects produced by a wide variety of agents to see whether any patterns emerge. Agents that bind irreversibly to DNA, such as nitrogen mustard and anthramycin, were found to cause the production of shorter RNA chains than normal. Bis-(chloroethyl)-nitrosourea (BCNU), sometimes considered an alkylating agents, did not produce this type of effect. Instead, it produced effects resembling those caused by less tightly bound compounds such as ethidium and ellipticine: the dominant effects are an increase in the average length of the synthesized nucleoplasmic RNA molecules and an inhibition of the processing of nucleolar RNA.

Several compounds related to BCNU were tested for ability to inhibit nucleolar RNA processing. The cyclohexyl analogue of BCNU (CCNU) and the fluoro-analogue of CCNU were active, whereas BCNU lacking the nitroso group was inactive, as were nitrosomethyl-urea and streptozotocin. It appears therefore that the 1-chloroethyl and 1-nitroso groups are required for processing inhibition.

The previous finding that agents of the DNA intercalating type inhibit nucleolar RNA processing, suggested that segments of helical RNA might exist at the processing sites of ribosomal precursor RNA. This lead to a study,

now in progress, of helical segments in various RNA fractions of L1210 cells.

3) Effects of chemotherapeutic agents on nuclear proteins in L1210 cells.

Because of the intimate relation between DNA and nuclear proteins, it is likely that these proteins will be affected by agents that act on DNA. In order to facilitate studies of this type, a new method was developed for the isolation and fractionation of nuclear proteins. This method has the advantage of minimizing spurious effects due to altered proteins in drug-treated cells. The major feature of the method is that the total nuclear proteins are collected and dissociated at an early stage of the procedure; the method does not rely on differential solubility properties which were found to be unreliable in drug-treated cells. The nuclear proteins are then fractionated chromatographically and characterized by amino acid composition. This method will be applied to the question of how DNA synthesis inhibitors affect the synthesis and nuclear incorporation of various nuclear proteins.

Serial No. NCI-6103
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Chemotherapeutic Agents on L1210 Cell Nuclear Proteins

Previous Serial Number: Same

Principal Investigators: Vincent H. Bono, Jr., and Robert Dion

Other Investigators: None

Cooperating Units: None

Man Years

Total:	2
Professional:	2
Other:	0

Project Description:

Objectives:

This project is concerned with the development of techniques for the analysis of drug effects on cell nuclear proteins and with their application to drugs having both primary and secondary effects on synthesis or function of nuclear proteins.

Methods Employed:

L1210 cells were grown in suspension cultures. Proteins were labeled by allowing growing cells to incorporate carbon-14 and/or tritium labeled valine and/or tryptophan. Cell nuclei were isolated by homogenization in a non-ionic detergent and centrifuged through sucrose. Nuclear proteins were fractionated by their acid solubility characteristics and also by methods to be described under major findings. Nuclear DNA and associated protein was obtained by centrifugation through NaCl-urea and then through alkaline CsCl. Isotopes were measured by standard methods of liquid scintillation counting.

Major Findings:

1) Development and characterization of a new method for the analysis of nuclear proteins by ion-exchange chromatography in 6M urea.

The nuclear proteins of isolated L1210 cell nuclei can be prepared in soluble

form by lysing in 2N NaCl, 7.5M urea and separated from DNA by ultra-centrifugation. From 96-98% of the nuclear protein is obtained in the supernatant fraction. When these proteins were applied to the urea chromatography system of Otaka et al. (J. Mol. Biol. 33:93-107 [1968]) which fractionates the basic proteins of bacterial ribosomes, they bound very tightly and failed to elute with high concentrations of sodium acetate, guanidinium chloride or Triton X-100 each in the presence of 6M urea. However, when they were applied to a column of IRC-50 (a carboxylate ion-exchange resin) equilibrated with 6M urea, 0.1M sodium phosphate (pH 6.8) and 0.3M guanidinium chloride, all of the proteins could be eluted in five large and two small peaks. The first peak (R.O.) consisted of protein which fails to bind to the column; three peaks (a, b and c) eluted in an 0.3 to 0.7M gradient of guanidinium chloride and a fifth peak (d) eluted in 4M guanidinium chloride; finally, two small peaks (e and f) eluted with 0.5N KOH and 1.0 M KCl.

As a first step in characterizing this urea-guanidine system, the chromatography of total nuclear protein was compared to that of the acid soluble proteins extracted from L1210 nuclei by 0.6N H_2SO_4 (pH 0.22). It was found that four peaks a, b, c and d consist primarily of acid-soluble protein and that three peaks R.O., e and f contain primarily acid-insoluble proteins. Peaks b and c are very low in tryptophan; peaks a and d contain small but significant amounts of tryptophan protein and the bulk of the tryptophan protein is found in R.O., e and f. Thus, the system separates total nuclear protein into three non-histone protein peaks (R.O., e and f) and into four histone peaks (a, b, c and d). The histone peaks a and d contain tryptophan which may indicate the presence of some non-histone protein in these fractions.

Table 1

% of Total Nuclear ^{14}C-Valine (Protein Labeled for 24 Hours)

	R.O.	a	b	c	d	e+f	Acid-insol.
Total N. protein	32.0	9.2	15.5	17.8	11.2	7.8	
Acid-sol. protein	5.0	6.4	12.9	15.2	8.5	4.1	47.9

% of Total Nuclear ^{3}H-Tryptophan

Total N. protein	61.6	5.4	2.2	2.1	4.6	17.1	
Acid-sol. protein	4.3	0.7	0.6	0.6	1.5	5.7	86.4

(a) The effects of various treatments of total nuclear proteins on their subsequent chromatographic behavior.

Neither their exposure to Ribonuclease A and Ribonuclease T_1 (in 2N NaCl, 7.5M urea under conditions that actively hydrolyse RNA) nor their exposure to H_2SO_4 at pH 1.0 (also in 2N NaCl, 7.5M urea) caused any change in the

chromatography of either the valine or the tryptophan labeled protein. These results indicate that neither RNA nor acid-labile bonds are responsible for the chromatographic character of proteins in this system.

(b) Studies on the rechromatography of the fractions.

Each fraction was prepared by chromatography of ^{14}C-valine, ^{3}H tryptophan labeled whole nuclear protein. Each fraction was mixed with unlabeled whole nuclear protein and rechromatographed. Thus, the chromatographic behavior of the labeled protein fraction was examined in the presence of a complete mixture of all the nuclear proteins. With the exception of peaks d, e and f, the major portion of each fraction rechromatographed with its initial elution characteristics. The proteins of peak d apparently interact with several other nuclear proteins and those of peaks e and f interact primarily with R.O. proteins.

Table 2

% of Total in Each Peak (^{14}C-Valine)

fraction rechromatographed	R.O.	a	b	c	d	e+f
R.O.	88	2	1	1	4	4
a	6	72	4	4	4	4
b	4		88		4	4
c	3	2	2	80	6	4
d	8	4	10	5	52	20
e+f	78				10	12

(c) Studies on the identity of the histone peaks a, b, c and d.

Whole calf thymus histone was prepared and chromatographed. Preliminary results of amino acid analysis of the four histone fractions indicate (using the noncenclature of Johns, Biochem. J. 92:(1964)55) that peaks a and b contain fractions f2a and f3, peak c corresponds to f2b and peak d corresponds to f1. Thus, the order of elution appears to be "argenine-rich," "slightly lysine-rich" and finally "lysine-rich" proteins. This is the same order of elution reported by Senshu (J. of Biochem. 67:[1970]473) using a non-aqueous system (a formic acid gradient in ethanol) on a carboxymethyl cellulose column. However, this order of elution is exactly the opposite to that obtained by Luck et al. (Biochem. 1:[1962]79) using a system which differs from the presently described urea-guanidine chromatography in that it lacks urea. A major advantage of the urea-guanidine system is that it allows the chromatography of a sample of total nuclear protein rather than requiring an acid-soluble protein subfraction.

(2) Studies on the effects of drugs on the synthesis of nuclear proteins

(a) Short term labeling of L1210 cells protein.

Using appropriately high concentrations of isotope, labeled valine and tryptophan are incorporated to significant extents at 1 hour by growing L1210 cell cultures. The results of chromatography of total nuclear proteins differ from those obtained after 24 hours of incorporation (see Table 1) in that peak d is relatively more highly labeled after 1 hour.

Table 3

% of Total Nuclear Isotope (1 Hour Incorporation)

	R.O.	a	b	c	d	e+f
Valine	29	12	15	17	19	8
Tryptophan	49	9	4	5	23	11

The ^{14}C and ^{3}H labeled form of each amino acid are incorporated equally well. In the following drug studies, one of a pair of L1210 cell cultures was treated with drug. Subsequently, ^{14}C-labeled amino acid was added to the control culture and ^{3}H-labeled amino acid was added to the drug treated culture and a 1 hour incorporation period allowed. Both cultures were mixed and various subcellular fractions prepared. The $^{3}H/^{14}C$ ratio of incorporated amino-acid in various fractions relative to the $^{3}H/^{14}C$ ratio of the mixed culture medium is a measure of the extent of drug effect, where a value of 1.0 indicates no effect.

(b) Effects of hydroxyurea and thymidine on the synthesis of cytoplasmic and nuclear proteins.

Both hydroxyurea and thymidine inhibit DNA synthesis and are capable of synchronizing certain cell lines including L1210. However, hydroxyurea is lethal to a fraction of L1210 cells while thymidine is not. The secondary effects of these drugs on the synthesis of cytoplasmic and nuclear proteins were examined to survey for differences. Hydroxyurea (1 mM for 60 minutes prior to labeling period) reduced the incorporation of valine into histone protein peaks to 14-33% of control while reducing incorporation into cytoplasmic and non-histone protein peaks to 60% of control. Incorporation of tryptophan was reduced to 55% of control in cytoplasmic protein and 45% of control in nuclear protein peaks. By contrast thymidine (5 mM either 10 minutes or 60 minutes prior to labeling period) had a more selective effect. There was no reduction of incorporation of valine into cytoplasmic or nuclear non-histone protein peaks while producing a reduction to 50-75% of control for the histone protein fractions. There was a uniform reduction in incorporation of tryptophan into both cytoplasm and nuclear protein peaks (80% of control.) These results are preliminary since, as yet there has been no careful correlation between them and the differing biological effects.

A more rapid, non-chromatographic system for the analysis of cellular

proteins was used to survey more broadly the effects of hydroxyurea, thymidine and several of the other deoxynucleosides. Nuclei were fractionated by extracting f1 histones with $HClO_4$ and the remaining histones with H_2SO_4. The effects of HU and TdR seen in the chromatographic system are evident in this survey method (Table 4). By contrast deoxycytidine inhibits all cellular protein synthesis except histone synthesis. This finding deserves further study.

Table 4

Effect of Incorporation of Valine

	No drug	HU	TdR	CdR
whole cell	1.10	0.77	0.92	0.80
cytoplasm	1.00	0.79	0.91	0.84
f1	0.95	0.27	0.49	1.15
histone (less f1)	1.04	0.41	0.58	0.99
non-histone	1.12	0.79	0.90	0.77

Since hydroxyurea produces a deficiency of all four deoxynucleotides, the relationship between this deficiency and the secondary effects on protein synthesis were examined by replacing one deoxynucleoside at a time in combination with hydroxyurea. It was found that the two deoxypurines selectively reverse the hydroxyurea effect on nuclear non-histone proteins.

Table 5

Effect of Incorporation of Valine

	HU alone	HU +TdR	HU +CdR	HU +AdR	HU +GdR
whole cells	.77	.70	.77	.82	.78
cytoplasm	.79	.72	.79	.86	.76
f1	.27	.14	.19	.28	.26
histone (less f1)	.41		.34	.41	.40
non-histone	.79	.64	.76	1.06	.92

(c) Effects of CCNU on cellular proteins.

An examination of the structure of the nitrosoureas suggests the possibility that they may be capable of cross-linking. Of particular interest was the possibility that they may cross link protein to DNA. With this in mind, the effect of 1 mM CCNU on the distribution of pre-labeled cellular protein into various subcellular fractions was examined. After a ten minute exposure to 1 mM CCNU, DNA synthesis is reduced to 2% of control and the cells develop cytoplasmic vacuoles at the cell membrane surface. At this time 5% of the cytoplasmic protein becomes bound to the isolated nucleus but there is no increase in DNA bound protein (protein sedimenting with DNA in alkaline CsCl

centrifugation). After a two hour exposure to CCNU 50% of the cytoplasmic protein is bound to the nucleus and this can be observed microscopically.

Proposed Course:

(1) Preliminary studies of the secondary effects on protein synthesis produced by drugs having primary effects on DNA synthesis indicate that further studies may be valuable. In particular, three questions should be pursued. (A) For a single cell type, different drugs having similar primary effects on DNA synthesis may have quite different effects on cell viability. Are there differences in secondary effects on cell protein synthesis that can be related to lethal effects on cells? (B) A single drug may be lethal to one cell type and not lethal to another. Are the secondary effects on the synthesis of nuclear proteins different in these different cell types? (c) What are the control linkages between replicative DNA synthesis and the synthesis of new nuclear proteins for the new DNA? Do the deoxynucleotides have a regulating effect on the synthesis of nuclear proteins?

(2) A survey of the secondary effects on protein synthesis of all kinds of chemotherapeutic drugs may yield valuable information about the kinds of mechanisms that link the primary drug effect to the irreversibly lethal effect on the cell.

Publication:

M. Colvin and V. H. Bono, Jr. The Enzymatic Reduction of Hydroxyurea to Urea by Mouse Liver. Cancer Research 30:1416-1519 (1970).

This report was previously entitled, Effects of Chemotherapeutic Agents on RNA Polymerase Reactions Primed by DNA and Nucleoprotein Templates.

Serial No. NCI-6104
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Chemotherapeutic Agents on RNA Metabolism*

Previous Serial No: Same

Principal Investigators: Herbert E. Kann, Jr., Zafar Iqbal, Lawrence Widerlite, and Kurt W. Kohn

Other Investigators: None

Cooperating Units: None

Man Years

Total:	4.5
Professional:	3.3
Other:	1.2

Project Description:

Objectives:

To examine a variety of chemotherapeutic agents, known to interfere with RNA synthesis, for selective effects on the patterns of RNA synthesis and processing. This information may help to determine the mechanisms of action of these agents and to classify these agents according to the effects produced. In addition, this study aims to utilize specific drug actions to elucidate the mechanisms of RNA synthesis and processing in the cell nucleus.

Methods Employed:

1) Growth of L1210 cells in suspension culture.
2) Incorporation of ^{3}H and ^{14}C RNA precursors; scintillation counting.
3) Cell fractionation and RNA isolation.
4) RNA sedimentation in sucrose gradients.

Major Findings:

1) Effects of drugs on RNA metabolism in L1210 cells.

The effects we have previously reported on the processing of nucleolar RNA in L1210 cells were extended to a series of compounds related to BCNU. The compounds were tested at concentrations of 50-100 µg/ml; 30 min. after

addition of compound, radioactive uridine was added, and after a 10-min. incorporation period, actinomycin was added to provide a chase period of 50 min. In control experiments, very little labeled nucleolar RNA remains after the actinomycin chase. Compounds that inhibit processing exhibit persistant labeled 45 and 32s RNA in the nucleolar fraction. Such inhibition was observed with BCNU, CCNU, and the fluoro analogue of CCNU. The following compounds were inactive: 1,3-bis(2-chloroethyl)-urea, N,N-nitrosomethyl urea, and streptozotocin. The structure-activity relation thus far indicates requirements for the 1-chloroethyl and 1-nitroso groups.

Structure activity studies are now in progress of the previously reported effect of BCNU on the sedimentation distribution of newly synthesized nucleoplasmic RNA.

2) Effects of drugs on helical RNA in L1210 cells.

Our previous finding that nucleolar RNA processing is promptly inhibited by the intercalating agents: proflavin, ethidium and ellipticine, and the demonstration that this effect is not secondary to inhibition of protein or RNA synthesis, suggested the possible existence of helical RNA regions which could bind intercalators and thereby prevent processing. This could occur if the specific sites of cleavage of the ribosomal precursor RNA molecules contained helical RNA segments.

In order to test this possibility, a study was begun of ribonuclease-resistant RNA in L1210 cells. Helical RNA has the property of ribonuclease-resistance at high ionic strength and sensitivity at low ionic strength. This type of resistance is presumptive evidence for helical RNA.

Experiments were carried out to determine how best to quantitate ribonuclease-resistance in various RNA fractions of L1210 cells. Two RNA-precipitating reagents were compared for their effectiveness in measuring undegraded RNA. Cetyltrimethylammonium bromide (CETAB) was judged superior to trichloracetic acid (TCA) in this regard, because of its ability to precipitate a larger fraction of partially degraded RNA, indicating that shorter resistant segments would be detectable. The results with CETAB also were more reproducible than those with TCA. The fraction of the RNA that is precipitable was measured as a function of incubation time with ribonuclease. A rapid fall in precipitability was followed by a slow decline. The latter was extrapolated back to zero time to define the ribonuclease-resistant fraction.

Previous work with known helical RNA of viral origin had shown that there is full resistance to ribonuclease at a salt concentration of 0.2 M. Since helical RNA of mammalian cells had not been well characterized, its sensitivity to ribonuclease was studied over a wide range of salt concentration. It was found that, unlike viral helical RNA, the ribonuclease-resistant RNA of L1210 cells shows a progressive increase in the amount of resistant material up to a NaCl concentration of approximately

1 M. This is perhaps due to imperfect matching of the complementary strands. The results were unchanged by the addition of $MgCl_2$. Studies are in progress with various ribonucleases, differing in ability to degrade helical RNA, as further test of the helical character of the L1210 RNA.

Patterns of ribonuclease-resistance versus NaCl concentration were determined for pulse labeled RNA of nucleoplasmic, nucleolar and cytoplasmic fractions. Characteristic differences were observed which are now under study.

Proposed Course:

1) Extend studies of effects on RNA synthesis patterns to other drugs. Refine methodology by adding RNA electrophoresis, and sedimentation in dimethylsulfoxide.

2) Compare ribosomal precursor RNA's in regard to ribonuclease-resistance patterns, in order to detect any differences attributable to helical RNA segments that might be destroyed during processing. Further characterize the helical segments by exclusion chromatography and electrophoresis. Determine the effects of drugs on the patterns of helical RNA synthesis.

3) Attempt to develop an in vitro system for the measurement of ribosomal precursor RNA processing, and then determine effects of processing inhibitors in this system.

Publication:

Alan L. Snyder, Herbert E. Kann, Jr., and Kurt W. Kohn, J. Mol. Biol. (1971) Vol. 58, in press. "Inhibition of the Processing of Ribosomal Precursor RNA by Intercalating Agents."

* This paper was previously entitled, Effects of Chemotherapeutic Agents on Nucleic Acids.

Serial No. NCI-6112 (New)
1. Lab. of Molecular Pharmacology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effects of Chemotherapeutic Agents on DNA

Previous Serial Number: None

Principal Investigators: Kurt W. Kohn, and Daniel Glaubiger

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.3
Professional:	1.3
Other:	0

Project Description:

Objectives:

To determine the mechanisms of interactions of chemotherapeutic drugs with DNA, and the consequences of such interactions on DNA structure and function.

Methods Employed:

1) Absorption spectroscopy; reaction kinetic measurements.

2) Viscometry.

3) Analytical ultracentrifugation.

Major Findings:

Interaction of anthramycin with DNA.

Our previous work had disclosed that anthramycin reacts highly specifically with DNA to form a firm complex, perhaps mediated by a covalent bond. These properties suggested that anthramycin may be used as a probe for the conformation of DNA in nucleoproteins as well as in complexes with other DNA-binding agents. In order to utilize anthramycin for this purpose, it is necessary to obtain more detailed information on the kinetics and specificity of the reaction and the structure of the complex.

a) Kinetics of the reaction.

The dependences of the reaction kinetics on pH and salt concentration were summarized in a previous report. It was hoped that measurements of reaction kinetics could be used to obtain information on the number and classes of binding sites in DNA. Detailed kinetic studies, however, disclosed that this approach is not feasible. Although at low extents of reaction the kinetics were first-order in anthramycin and DNA, it was found that at higher extents of reaction there is a component of the kinetics that depends explicitly on time. This might be caused by a slow rearrangement of the complex, resulting in altered reactivity. This is an interesting and unusual feature of the reaction, but it interferes with our initial objective of utilizing kinetic measurements to determine properties of DNA in structures such as nucleoproteins. In order to obtain this type of information, it will therefore be necessary to make measurements near the range of binding-site saturation. This approach is subject to the limitation that when DNA (or nucleoprotein) is extensively reacted with drug, its structure may be grossly altered, so that what is measured may not directly reflect the original structure.

b) Specificity of the reaction.

In order to determine the types of nucleotide arrangements that can serve as binding sites, synthetic polynucleotides of known structure were tested for anthramycin-binding capacity. It was found that poly dG and poly dG:poly dC have anthramycin binding capacities comparable with that of DNA.

By contrast, the following were found not to bind anthramycin: poly rG, poly rC, poly rG:poly rC, poly dC, poly dA, poly dT, poly d(AT), and poly dA:poly dT. The results thus far suggest (but do not prove) that anthramycin reacts with G. The marked difference between the behaviors of ribo-versus deoxyribo-polymers must have an important bearing on the structural requirements for reaction, but its significance is not clear at present. Studies are now in progress to determine binding to hybrid complexes such as poly dG:poly rC and poly rG:poly dC.

Specificity for reaction with DNA in cells was determined by reacting L1210 cells with large concentrations of anthramycin. The DNA was isolated from various cell fractions and purified using sodium lauryl sulfate, pronase, phenol, and alcohol-precipitation. The maximum amount of firm anthramycin binding to DNA that could be achieved was about 25% of that attainable by reaction with pure DNA. The anthramycin recovered firmly bound to DNA accounted for at least 70% of the amount of anthramycin retained in the washed cells. Most of the remainder of the intracellular anthramycin was accounted for in the soluble fraction and was completely dialysable, indicating that this anthramycin is unbound or loosely bound. These results indicate that this anthramycin is unbound or loosely bound. These results indicate specificity of anthramycin for binding to DNA in intact cells. It was also found that the extent of reaction with cell DNA increases for a period of hours in the presence of anthramycin. The

reaction with intracellular DNA is therefore gradual and progressive for several hours.

c) Structure of the complex.

Previous work had disclosed a marked increase in the viscosity of DNA at high extents of reaction with anthramycin. Up to the half-saturation point, however, there was little change in viscosity; the viscosity rise occurred only as the DNA approached saturation with drug. In order to explore the origin of this unusual behavior, sedimentation and viscosity measurements were performed on un-sonicated and sonicated anthramycin-DNA complexes of various drug:DNA ratios. The rise in viscosity was found to be accompanied by a slight fall in sedimentation constant. This rules out end-to end aggregation as a cause of the increased viscosity. In the case of sonicated DNA, the results were qualitatively similar, but smaller in magnitude. The most likely interpretation is that anthramycin stiffens, but does not increase the length of the DNA helix.

Proposed Course:

1) Test the inference that anthramycin stiffens the DNA helix by light scattering measurements. Carry out further studies with synthetic polynucleotides to further define the requirements for binding.

2) Determine the binding capacity of anthramycin-saturated DNA for other DNA-binding agents (e.g. actinomycin) in order to determine whether there is overlap between classes of binding sites for different agents. This could lead to a mapping of DNA sites corresponding to the different agents.

3) Measure the anthramycin-binding capacity of chromatin in order to determine what fraction of the binding sites of DNA are accessible in chromatin structures.

Publication:

Kurt W. Kohn and Carlos L. Spears. Reaction of Anthramycin with DNA. J. Mol. Biol. (1970) 51:551-572.

ANNUAL REPORT

LABORATORY OF TOXICOLOGY

CHEMOTHERAPY

NATIONAL CANCER INSTITUTE

July 1, 1970 -- June 30, 1971

During the past year the Laboratory of Toxicology has continued to streamline its chemotherapy-program-support role (preclinical toxicology), and also to strengthen its independent basic research program.

The standard preclinical toxicology protocol used by this laboratory has been updated to provide information concerning the toxic potential of new drugs for both dogs and monkeys prior to Phase I clinical trial. The Decision Network Stage III has also been revised to reflect these changes. Each senior staff member of the Laboratory has been assigned definite responsibilities in the preclinical toxicology area. This role includes the supervision of contracts, continual monitoring of the progress of new agents during preclinical studies, and reporting on individual drugs at Operating Committee, Experimental Therapeutics, and Decision Network meetings. In this way, a specified scientist is individually responsible on a day-to-day basis for each new agent in preclinical development. These contracted studies are summarized in the Appendix. In addition to the routine preclinical toxicology, a number of more basic research efforts have been undertaken to explore in greater depth the mechanisms of acute and chronic drug-induced disease.

The future development of the Laboratory of Toxicology is dependent on an ability to recruit outstanding scientists with expertise in the various areas of toxicology (pharmacology, physiology, biochemistry, and pathology) who are willing and able to support the Chemotherapy program and at the same time conduct an outstanding independent basic research program. Along these lines, we were very fortunate to have Dr. David M. Young join the Laboratory last fall, and recently have offered a position to another very able comparative pathologist who will join us later this spring.

The ability of preclinical toxicology to predict the quantitative and qualitative toxicities of a new drug in man continues to be a major interest of this laboratory. This retrospective evaluation of preclinical toxicology for its clinical relevance allows us to continually strengthen this effort. All members of the Laboratory have been active in this undertaking, especially Dr. Elton R. Homan and Dr. David M. Young.

Independent projects of each senior staff member continue to contribute to the basic understanding of drug-induced toxicity. It is obvious that more information is needed to determine and understand the mechanisms of drug-induced hazards. This knowledge will hopefully allow for the future control of toxicity with a resulting increase in clinical efficacy of new and currently used antineoplastic agents.

Dr. Homan's major effort has been in the area of data analysis. He is currently evaluating the quantitative comparison between the toxicity of anticancer agents in preclinical species (dogs and monkeys) and man. This work will allow for a more rational extrapolation of the initial Phase I clinical dose from the preclinical studies.

Dr. David A. Cooney has continued his fine work in the area of biochemical toxicology with special emphasis on a variety of aspects concerning L-asparaginase. These studies include the development of sensitive methods for the detection of picomole quantities of L-aspartic acid and L-asparagine. This radiometric technique has been used to serially measure the concentration of L-asparagine in mouse blood and to assess the influence of tumor growth and immunity on the kinetics of this amino acid. A colorimetric technique for measuring L-asparagine in acrylamide and other gels has also been developed; it was found that peroxydisulfate, the catalyst for the polymerization of acrylamide, irreversibly inactivated L-asparaginase at the concentrations used. A large number of agents were tested both in vitro and in vivo for their ability to inhibit L-asparagine synthetase. The effect of azotomycin and azaserine on amino acid metabolism is being studied; azotomycin is being used clinically in combination with L-asparaginase. The toxicity and biochemistry of L-asparaginase from Erwinia carotovora and E. coli have been compared and contrasted. L-Asparaginase has been covalently coupled to Dacron grafts, and the enzymic properties of the bonded enzyme have been studied. Other studies are also in progress.

Dr. David M. Young has initiated an independent research program to help define the pathogenesis of drug-induced diseases utilizing biochemical, cytochemical, and morphological methods. He is also working closely with the Laboratory of Pathology (GL&C,NCI) to evaluate the ability of preclinical studies to predict drug-induced histopathologic alterations in man. Initial studies involve the following drugs: Bleomycin (NSC-125,066), Daunomycin (NSC-82151), and the Nitrosoureas (NSC-409,962, 79037, 95441). In addition, other studies concern the effects of Streptozotocin on parathyroid hormone and calcitonin, the pathogenesis of L-asparaginase-induced hypocalcemic tetany in rabbits, the interrelationships of calcitonin and histaminase in medullary carcinoma of the thyroid gland, the effect of cancer chemotherapeutic agents on lymphoreticular (immunogenic) tissues, the toxicity of hormonal antineoplastic agents, the pathologic evaluation of spontaneous leukemia in AKR mice, and the role of altered mineral metabolism in Daunomycin-induced cardiotoxicity.

Dr. Robert L. Dixon and Mr. Insu P. Lee have continued to study the effect of antineoplastic agents on spermatogenesis in male mice. These studies involve both in vivo serial mating, and in vitro sedimentation velocity separation of spermatogenic cell types. The incorporation of thymidine, uridine and L-leucine by specific cell types, and the effect of five anticancer agents on the incorporation of these nucleosides and amino acid have been extensively studied.

Dr. Aron Primack and Dr. Dixon have undertaken the study of various pharmacologic and toxicologic aspects of the clinically useful alkylating agent, cyclophosphamide. The interaction of potassium iodide and cyclophosphamide has been studied in mice; potassium iodide increases the toxicity of cyclophosphamide, but has no effect on efficacy. Bladder irrigation with sulfhydryl containing compounds (cysteine, Mucomyst) has been demonstrated to ameliorate the drug-induced cystitis associated with high doses of cyclophosphamide. The metabolites of cyclophosphamide have been separated by column chromatography using L-20 Sephadex. A comparison is being made of the metabolites produced by in vitro acid hydrolysis and in vivo enzymic alterations. Further, comparisons of metabolism in various animal species are being made, the effects of other therapeutic agents on cyclophosphamide activation are being studied, and the antitumor activity of the isolated metabolites is being investigated.

Mrs. Ruth Davis has continued her outstanding work in all aspects of the laboratory's function. Her efforts in the preclinical toxicology area should be specifically acknowledged. The Laboratory's secretaries, Mrs. Audrey Loveridge and Mrs. Donna Smith are also outstanding, and extremely important to the efficient functioning of the Laboratory of Toxicology.

Serial No. NCI-3507
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Preclinical Toxicology of Drugs Potentially Useful in Cancer Chemotherapy

Previous Serial Number: Same

Principal Investigator: Elton R. Homan

Other Investigators: Thomas Cameron, Ruth Geran

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Others:	0

Objectives:

The objectives of this project are 1) to evaluate the toxicologic and pharmacologic properties of potential cancer chemotherapeutic agents in animals for the purpose of expediting clinical trial, 2) to develop better predictive models for the study of toxic effects of chemotherapeutic agents, 3) to study the influence on drug toxicity of dosage scheduling, concomitant drug administration, and alteration of environment variables, and 4) to facilitate the handling and use of toxicologic data generated by the laboratory and its contractors by development of data processing methods.

Principal Findings:

Maximum Tolerated Doses of Antitumor Agents: Correlation Between Experimental Animals and Man

The predictive value of animal toxicity studies of cancer chemotherapeutic agents was examined by considering the quantitative relationships between maximum tolerated doses (MTD's) established in preclinical studies using dogs and monkeys and in clinical investigations. The logarithms of mg/kg MTD's of 37 compounds in three species and the logs of MTD ratios were found to be normally distributed. There is an approximate equivalence between human and dog mg/kg MTD's (geometric mean MTD ratio equals 0.94) but monkey mg/kg MTD's are significantly greater than human MTD's (geometric mean MTD ratio equals 0.53; $p < 0.01$).

The distribution of the logs of the MTD ratios of alkylating agents and purine and pyrimidine antimetabolities (Group I) was compared with the distribution of log MTD ratios of antibiotics, chelating agents, steroids and others (Group II). The variability of Group I data is considerably less as indicated by the coefficients of variation, possibly indicating a greater overall homogeneity of response to these drugs. For Group I drugs as well as for the whole sample, the dog MTD is a more accurate predictor of the human MTD than is the monkey MTD when doses are compared on the basis of mg/kg.

The work of Freireich et al indicates that for certain antitumor agents evaluated in several animal species from mouse to man, the use of body surface area provides better dosage equivalence in toxicity studies than body weight (e.g., mg/sq.m rather than mg/kg). The present study involved data from only monkeys, dogs and man and included data from a wider spectrum of antitumor drugs. Within this group of animals of relatively similar body size, the predictive superiority of mg/M2 was not apparent. The variability of the MTD ratios emphasizes the need for considerable care in extrapolating from animal MTD's.

It is a current practice to suggest an initial clinical dose of antitumor drugs as 1/10 of the animal MTD (expressed in mg/kg). The data developed and conventional statistical assumptions permit calculation of the probability of exceeding any dosage ratio. This probability, which may be thought of as the clinical risk associated with any predicted dosage level, varies with the predictive species and the assumptions regarding dosage equivalency. Prediction from dog studies of an initial clinical dose apparently bears a smaller risk than that associated with monkey studies. The smaller risk associated with the mechanistically more homogenous drug Group I implies that variability among relative responses is not random, but can be attributed to rational causes.

Excretion of Indocyanine Green (ICG) by Laboratory Animals

Indocyanine green, or ICG, an organic anionic dye originally introduced as a means of evaluating cardiac function by the dye dilution technique, has properties which also commend its use as a means of examining liver function. It is rapidly removed from the plasma solely by the liver, and is not metabolized. It does not undergo entero-hepatic circulation, is non-irritating and has a very low order of toxicity. It has an absorption maximum at about 800 nanometers, well away from interference by hemoglobin.

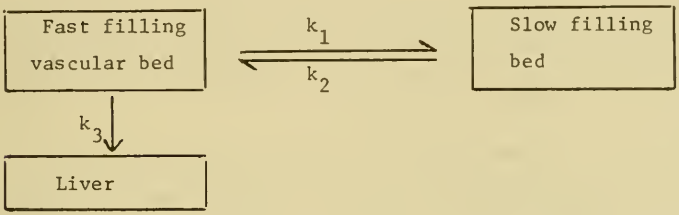
Work on the use of ICG excretion as a means of evaluating hepatic function has been continued and extended to consideration of compartmental analysis of the data. In this model, dye is transferred from one compartment to another at a rate which is proportional to the concentration in the source compartment at any time (first order kinetics). Thus at the moment of injection all the dye is in the plasma compartment and is transferred to the liver at a rate equal to the product of the plasma concentration and the transfer constant. The dye which enters the liver compartment is then subject

to the two removal processes which transfer the dye back into the plasma and into the bile at rates which are proportional to the respective transfer constants. The algebraic notation used here and the analytical procedures are adapted from the work of Richard and co-workers on the transfer of BSP.

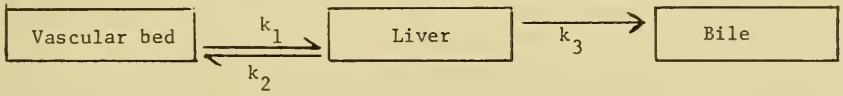
The solution of the linear differential equations which express these relationships leads to the double exponential equation for total dye concentration in the plasma. The numeric values of the transfer constants can be obtained from the coefficients of the exponential relationships.

Experiments will be continued to determine the ability of the ICG test to detect minimal liver injury from known hepatotoxins. Preliminary results have been obtained for carbon tetrachloride, cyclophosphamide and Saramycetin.

The double exponential equation which has been used to describe ICG excretion in mice has been further examined in terms of possible underlying physiologic kinetics. At least two compartmental models would appear to constitute valid bases for the observed relationships. One of these involves two vascular beds and has the configuration:



The other model hypothesizes a somewhat different compartmental configuration:



As indicated above mathematical analysis of both compartmental models leads to similar time-concentration decay curves, thus other means must be used to distinguish between the models. One technique of experimentally distinguishing between these two alternatives is perfusion of isolated liver and such studies are anticipated when equipment is available.

In pursuance of more accurate and convenient evaluation of liver function in toxicologic studies, studies have been undertaken in cooperation with Dr. Aron Primack to determine the feasibility of continuous in vivo monitoring of ICG in Rhesus monkeys and Beagle dogs. Preliminary results to date have demonstrated that the methods developed are applicable to these species. Anticipated difficulty due to interference with the optical system of the sensor by pigmentation of the animal's skin has not been observed.

Absence of Effect by Tetraethyl Thuiram Disulfide (Antabuse) on Cyclophosphamide Lethality in Swiss Mice

Experimental data from Southern Research Institute (SRI) indicated the appearance of an anionic metabolite, attributed to aldehyde oxidase activity, following administration of cyclophosphamide. Antabuse is a potent inhibitor of aldehyde dehydrogenase and of other oxidative enzymes conceivably involved in cyclophosphamide metabolism. If the dehydrogenase or xanthine oxidase (another Antabuse susceptible enzyme) were responsible for production of metabolites which played a significant role in the effects of cyclophosphamide, it might be anticipated that prior administration of the inhibitor would decrease lethality following administration of cyclophosphamide.

To test this hypothesis, the influence of two daily doses of 300 mg/kg Antabuse, orally, on lethality of cyclophosphamide (240 - 408 mg/kg - I.P.) was examined. The effect on toxicity of 25% ethanol (2.5 and 10 ml/kg - P.O.) was examined concomitantly as a positive control. No discernible effect was observed on lethality of mice following cyclophosphamide or ethanol although the CNS depressive effect of ethanol was markedly prolonged by Antabuse. It is concluded that there is probably no major contribution to cyclophosphamide toxicity by Antabuse susceptible enzyme systems. Since the work was completed, it has been reported by SRI that aldehyde oxidase *in vitro*, but not xanthine oxidase, was active in production of the metabolite, which was therapeutically inactive in animal tumor tests.

Influence of Chemotherapy on L-Asparagine Clearance in Canine Lymphoma

Investigations begun during the previous year involved the study of canine lymphoma as a model for chemotherapy. During the current year, this work has been continued and extended to include an examination of the clearance of labelled L-asparaginase. This study was carried out at the suggestion of and in cooperation with Dr. David Cooney and Dr. Thomas Cameron.

The radioactivity associated with 4-^{14}C-L-asparagine was measured in plasma samples taken at intervals of 10 to 210 minutes after intravenous administration of 20 microcuries to a Great Dane dog bearing a lymphoma with a tumor mass of over one kilogram before institution of therapy. Studies were conducted prior to therapy, during cyclophosphamide induced remission, during relapse, and during successive L-asparaginase induced remissions.

The removal of radioactivity from plasma was evaluated by graphic analysis of the semilog plot of specific activity versus time. Two exponential terms were required to satisfactorily characterize the observations. Half-time for the fast and slow phases and the volumes of distribution were calculated from the fitted lines.

Removal of 4-^{14}C-L-asparagine from plasma was markedly faster during florid tumor growth than during remission, suggesting utility of this parameter as an estimate of L-asparaginase therapy. The shortest $t_{1/2}$ observed was during tumor regrowth following L-asparaginase induced remission. It may be significant that subsequent L-asparaginase chemotherapy was unsuccessful. There was

a definite positive correlation between tumor mass and the volume of distribution of L-asparagine, and possibly between tumor rate of growth and volume of distribution. The available data are inconclusive in regard to the latter point, which deserves greater attention.

Computer Generation of Semiquantitative Toxicologic Data Summaries

Examination of detailed tabulations of hematologic and chemical alterations in an attempt to render digestible the information contained therein suggests that such data can be usefully summarized in terms of the maximal change of each parameter. The reference point for these changes may be laboratory mean values in the case of those parameters which exhibit small variation independent of drug treatment, or alternatively, individual animal pre-treatment values for those parameters which tend to vary more widely under normal conditions. Dr. U. Schaeppi of Mason Research Institute has developed criteria for scaling such toxic manifestations on the basis of a range of 0 to +4 or 0 to -4. Summarization then becomes a mechanical task of comparing the maximum and minimum observed values of each parameter with the appropriate range limits.

Such a procedure is susceptible to computerization and a program has been written which generates tabular summaries of semiquantitative toxicity ratings from input data consisting of animal and parameter identification and maximum and minimum values of each parameter. A significant advantage of this approach is the fact that the program can be run by non-professional personnel without training in the evaluation of data. The program incorporates a dictionary of synonyms which was developed for the names and abbreviations of parameters, so it is not necessary to conform to a rigid nomenclature or sequence in entering data. It has been shown that the use of this program can facilitate the comparison of doses, regimens, and agents in toxicologic evaluation.

Comparison of data from several laboratories discloses significant differences between normal control values of the various laboratories. It will be necessary to compensate for these differences in calculating semiquantitative toxicity ratings by either programming the individual laboratory means or by reliance on pretreatment values as reference standards.

Development of a Stochastic Model of Toxicologic Investigation of Antitumor Drugs

In the design and performance of toxicologic studies, numerous assumptions are made in the extrapolation from available data to the dose-response relationships which are the intended goal. These assumptions may be implicit or explicit and in the absence of adequate data require more or less arbitrary decisions based on intuition or experience.

The purpose of this study is to examine the relationships between assumptions made in the performance of toxicologic investigations and the decision making processes by the investigator as they influence the outcome of such work.

A stochastic empirical model of the procedures has been developed which simulates performance of a single dose toxicity study. Interspecies equivalency factors, the log dose-probit response relationship, the bimodal time-response curve, and normal distribution of variates are concepts incorporated in the model.

In this model, in which an investigator interacts with a computer oriented simulator, the individual is presented with results of a hypothetical mouse lethality study and then designs sequential studies with the purpose of identifying the parameters of a single dose dog LD_{50} investigation as in the real situation. After entering each study design, the investigator is presented with a table showing, for each animal, the "observed" toxicity rating (0 - 4 or dead) on each day of observation of the study design. This cycle can be repeated as many times as desired.

Using the model it is possible to determine the influence of such decisions as frequency and timing of observations, dosage levels used, number of animals per dosage level and number of dosage levels in each sequential study on the efficiency of study design in arriving at conclusions. Since it is possible to determine the cost of the various elements of a toxicologic study, a secondary benefit is information on the economic efficiency of various study designs.

Significance to Bio-Medical Research and the Program of the Institute

The limitation imposed on cancer chemotherapy by clinical toxicity requires a continuing search for appropriate experimental animal models. Two complementary goals are involved in this search: 1) improved systems for predicting toxicity and 2) means of circumventing or ameliorating toxic effects. Implicit in both of these is the need for an understanding of pertinent physiological mechanisms which permits rational extrapolation.

Thus, the value of the studies carried out under this project to the larger program is twofold. In addition to providing toxicologic data on specific antitumor agents of present or potential clinical interest, the studies are intended to develop tools for subsequent investigation which are more sensitive or predictive.

With regard to data processing activities, there is increasing awareness in the toxicology community at large, as well as in the Laboratory of Toxcology, of the need for more effective ways of handling and presenting toxicologic data. The work being performed is intended to develop the capabilities of the laboratory in the area of data processing and to determine the extent to which contractors can participate effectively in these activities.

Proposed Course:

Structure Activity Relationships of Hydrazine Compounds

Studies have been initiated with Mr. Insu Lee to examine the dose response relationships of hydrazine analogs, including Procarbazine, with respect to

hepatotoxicity as reflected in such effects as pentobarbital sleeping time, metabolism of ^{14}C-pentobarbital, and organic dye excretion. Preliminary results have shown that hydrazine analogs produce drug and dose related alterations of spontaneous activity of mice, pentobarbital sleeping time, and acute lethality. MIH produces a significant prolongation of ^{14}C-pentobarbital clearance from the plasma of mice. It was noted that the commercial preparation of ^{14}C-pentobarbital used was not chromatographically pure and further work will be undertaken pending availability of a chromatographically homogenous preparation of this agent.

Interaction Between Halothane and Antitumor Drugs

Data has been published by Bruce and co-workers (1966, 1969) indicating that prolonged administration of halothane has an inhibiting effect on DNA synthesis of gut and marrow. This inhibition has been shown to reduce the sensitivity to cytosine arabinoside of normal mouse hematopoietic cells but not lymphoma cells. In this laboratory, studies are under way in cooperation with Miss Ruth Geran to determine whether this difference can be exploited to protect animals from the toxicity of antitumor drugs while maintaining therapeutic effectiveness. Preliminary findings include the observation that there is considerable diurnal variation in the level of anesthesia of mice associated with constant concentrations of halothane.

Development of Methods for the Evaluation of Hepatotoxicity

The results of ICG studies leave unanswered the question of the interpretation of the compartmental analysis in terms of physiologic parameters. It would be valuable to determine whether the results of this test are in fact reflections of specific hepatic functions or include superimposed vascular alterations. It is anticipated that the performance of indocyanine green removal studies by isolated perfused liver should provide answers to these questions.

Possible Antigenicity of 5-Azacytidine

The clinical formulation of 5-azacytidine incorporates the drug with polyvinyl pyrrolidine (PVP - Mol. wt. $\sim$ 15,000). The suggestion has been made that this combination could prove antigenic in patients. This possibility is being examined by conventional guinea pig sensitization.

Publications:

1. Cooney, D. A., Davis, R. D., Homan, E. R., Rakieten, N., Schaeppi, U. and VanAtta, G.: Studies on the Inhibition of Clearance of Organic Dyes by Saramycetin. British J. Pharm: 41, 140 (Jan.1971).

2. Adamson, R. H., Fabro, S., Homan, E. R., O'Gara, R. W. and Zendzian, R.P.: Pharmacology of Polyriboinosinic: Polyribocytidylic Acid, A New Antiviral and Antitumor Agent. Antimicrobial Agents & Chemotherapy, 148-152, 1969. Editor: G. L. Hobby.

3. Hill, D.A., Baron, S., Levy, H.B., Bellanti, J., Buckler, C. E., Cannellos, G., Carbone, P., Chanock, R. M., DeVita, V., Guggenheim, M.A., Homan, E., Kapikan, A. Z., Kirchstein, R. L., Mills, J., Perkins, J.C., Van Kirk, J. E., Worthington, M., and Zendzian, R. P.: Clinical Studies of Induction of Interferon by Polyinosinic Polycytidylic Acid. Perspectives in Virology, Vol 7, 197-222, Ed.: Morris Pollard, Academic Press.

Serial No. NCI-3518
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: L-Asparagine; synthesis, biological role and hydrolysis

Previous Serial Number: Same

Principal Investigator: David A. Cooney

Other Investigators: Ruth D. Davis, Harry Milman

Cooperating Units: South Shore Analytical and Research Lab.,Inc. Islip,N.Y.;
Mason Research Institute, Inc., Worcester, Mass.;
Corning Glass Co., Corning, N.Y. - Howard Weetall;
Yale University School of Medicine - Dr. R. Handschumacher;
Southern Research Institute - Dr. Robert Pitillo;
Arthur D. Little, Inc. - Dr. Robert Liss;
National Cancer Institute, Latvian Academy of Sciences - Dr. Raimonds Zhagats

Man Years:

Total:	1 1/2
Professional:	1/2
Others:	1

Objectives:

Because L-asparaginase continues to be a drug of clinical interest, during the past year investigations into the toxicologic and biochemical properties of the enzyme have continued. The following projects were undertaken or completed in the course of the year:

1. A radiometric technique for measuring picomole quantities of L-aspartic acid and L-asparagine was developed. In the presence of 1-^{14}-C-α-ketoglutaric acid and L-glutamic oxalacetic transaminase (GOT), L-aspartic acid will be transaminated according to the reaction:

L-aspartic acid + 1-^{14}-C-α-ketoglutaric acid $\xrightarrow{\text{GOT}}$ oxalacetic acid + 1-^{14}-C-L-glutamic acid

If the oxalacetic acid is removed continually with Malic acid dehydrogenase and DPNH, the yield of radioactive L-glutamic acid will be stoichiometric to the amount of L-aspartic acid present originally. All unreacted α-keto-glutaric acid is quantitatively α-decarboxylated with 1% H_2O_2 in 1 N HCl,

the reaction mixtures are heated at 90°C until dry, and the residue counted directly in a scintillation spectrometer. Twenty picomoles of L-aspartic acid or L-asparagine can be easily and reproducibly quantitated in this way.

2. The radiometric technique described above has been used serially to measure the concentration of L-asparagine in 1 µl aliquots of mouse blood. In addition, the influence of the growth of the L5178Y tumor, (both the L-asparaginase-sensitive (AS) and-resistant (AR) variants) on the plasmatic levels of L-asparagine has been studied. Unlike the 6C3HED-AS lymphoma, which depresses the level of circulating amide as it grows, the L5178Y tumor fails to alter circulating L-asparagine significantly even at a time when the net tumor volume is 1 gram.

3. The radiometric technique was also applied to studies which were carried out in conjunction with Dr. John Glynn, CCNSC, C, NCI, on the role played by the immune state to L-asparaginase in the clearance of challenge-doses of L-asparaginase. Since these studies are described in full elsewhere in this report, the results will only be sketched-in here.

(a) Doses of L-asparaginase ordinarily curative in normal mice, when given intravenously to immune mice are without curative effect.

(b) L-Asparaginase is cleared from the plasma of normal mice with a half-life of 2-3 hours and from the plasma of immune mice with a half-life of 2-3 minutes.

(c) Ten IU of L-asparaginase given to a normal mouse reduces the circulating L-asparagine to zero for at least twenty-four hours. In the immune mouse the state of enzyme-induced L-asparaginopenia lasts only 7-8 hours.

4. A colorimetric technique for measuring L-asparagine in acrylamide, and other gels, was developed. L-Asparaginase in the gel is used to generate L-aspartic acid which is then transaminated with GOT to yield oxalacetic acid; the oxalacetate will couple with a suitable diazonium salt to yield an intense red chromogen. The salt used most frequently has been 6-benzamido-4-methoxy m-toluidine diazonium chloride. Using this technique it has been possible to demonstrate that five of the eight satellite bands seen when L-asparaginase is electrophoresed in polyacrylamide gels exhibit amido-hydrolytic activity. Attempts to apply the technique to the tissue of normal and immune mice receiving the enzyme parenterally, have failed.

5. The Inhibition of L-Asparaginase by Peroxydisulfate.

These studies were carried out in conjunction with Mr. Leon Moore, CTEB, C,NCI. Ammonium peroxydisulfate is the most commonly used catalyst for the polymerization of acrylamide. But when the colormetric staining technique described above was applied to gels which had been cross-linked with this oxidant, low color-yields were obtained. This phenomenon was traced back to the peroxydisulfate used in preparing the gels: it was found irreversibly to inactivate L-asparaginase. Thus, 50% inhibition results when

L asparaginase is even briefly exposed to 5×10^{-3} M ammonium or potassium peroxydisulfate. The enzyme so exposed has been digested enzymatically with the following sequence of proteases in the presence of mercaptoethanol; trypsin, pronase, subtilisin, carboxypeptidases A & B; the digests were analyzed on the Jeolco amino acid analyzer. It was found that the L-cysteinyl residues were partly oxidized and that 80% of the L-methioninyl residues of L-asparaginase were deleted by exposure of the enzyme to the catalyst. Thus oxidation of sulfur functions appears to be the principal mechanism for the subject inhibition. Since many powerful SH blocking agents failed to inhibit L-asparaginase, it is likely that oxidative destruction of L-methioninyl residues has inactivated the enzyme.

6. A systematic search has been made for inhibitors of L-asparagine synthetase. Over 85 compounds have so far been tested against the crude synthetase from the L5178Y-AR tumor developed at NCI by Mr. Ned Greenberg. The following agents were found to be strong inhibitors of the enzyme: maleimide and other sulfhydryl reagents, such as PCMB and ethacrynic acid (effective at concentrations down to 5×10^{-5} M); homologues of L-aspartic acid, such as amino-malonic acid, (effective at concentrations down to 5×10^{-4} M); and product-analogues, such as L-asparaginol, (effective only at concentrations greater than 1×10^{-3} M). The search for agents which can irreversibly inhibit this synthetase at low (pharmacological) concentrations is continuing.

7. A study on the influence of parenteral administration of inhibitors of L-asparagine synthetase on the circulating L-asparagine of the mouse was begun.

Since a twenty-four hour elimination of L-asparagine from the circulation appears to be a pre-requisite for oncolysis, attempts have been made to deplete the plasmatic L-asparagine by simple pharmacologic means. Last year it was reported that the agent, CONV, chloro-oxo-norvaline (NSC-124,412) was the most potent general antagonist of L-asparagine synthesis available. This agent has therefore been given intravenously to BALB/C mice. After doses of 100 mg/kg ($\cong LD_{20}$) the concentration of L-asparagine falls to one half of its normal level for at least three but not for twenty-four hours. Similar experiments with amino malonic acid, the next lower homologue of L-aspartic acid are also underway.

8. The influence of Azotomycin (NSC-56654) on amino acid metabolism in the murine liver has been studied. Single large doses of Azotomycin (a drug being again used clinically in combination with L-asparaginase) have been found to elevate the concentration of L-glutamine in mouse liver by 300% for periods in excess of 72 hours after dosing. The livers of treated mice also became fatty. Thus the utilization of L-glutamine is impaired more than its synthesis. However, L-glutamine is not the only amino acid whose homeostasis is perturbed by the antagonist of L-glutamine. The concentrations of L-aspartic acid, L-alanine and L-glutamic acid are all seriously altered by treatment with Azotomycin and the duration of the biochemical defect is as long as that involving L-glutamine. Studies of the principal enzymes utilizing or producing L-glutamine, L-alanine, L-glutamic acid and L-aspartic

acid in the face of large single and small repeated doses of Azotomycin are therefore underway.

9. Azaserine is another typical antagonist of L-glutamine. In contrast to Azotomycin however, parenteral Azaserine failed to elevate or depress the hepatic concentration of L-glutamine or of L-asparagine in the mouse. This finding is taken to mean that the antagonist poisons the pathways of input and utilization of L-glutamine to about equal degrees.

10. Maleimide was last year reported to be a substrate for L-asparaginase. In the present year maleimide has been shown to inhibit the in vivo biosynthesis of L-asparagine by the L-asparaginase resistant L5178Y tumor for at least twenty-four hours. In some mice the inhibition was total at 1 day after 8 mg/kg of imide. No evidence of oncolysis was seen either in this L-asparaginase-resistant tumor or in its sensitive counterpart; nor could maleimide make the L5178Y-AR tumor sensitive to the oncolytic action of L-asparaginase. In the present work-term it was also demonstrated that the L-asparaginase from Erwinia carotovora has been found to catalyze the imidohydrolysis of maleimide.

11. L-Asparaginase from Erwinia carotovora has been studied from a toxicologic standpoint and compared to the coliform enzyme. The following differences have emerged from this study; the E. coli enzyme, as mentioned, is floridly steatogenic to Rhesus liver when given for five days at doses of 50 - 100 IU/kg/day. The Erwinia enzyme is steatogenic, but only very mildly so, and at doses of 2,000 - 10,000 IU/kg/day x 5 days. The coliform enzyme depresses hepatic L-asparagine whereas the Erwinia enzyme does not; the coliform enzyme is cleared from Rhesus plasma slowly (mean t 1/2 approximately 12 hours); the Erwinia enzyme is cleared rapidly; neither enzyme consistently alters the concentration of hepatic L-glutamine; neither enzyme depresses the cerebral L-asparagine or L-glutamine of mice given large single doses.

12. The influence of Emetine on protein synthesis was studies in the squirrel monkey. After single doses of Emetine or dehydroemetine given intravenously, protein synthesis as adjudged by the incorporation of ^{14}-C-L-valine into plasma proteins was sharply curtailed for about one hour. These doses of the alkaloids were acutely but transitorily cardiotoxic and it was suggested that the synthesis of membrane proteins necessary for normal electrical conduction was being inhibited.

13. L-Asparaginase has been covalently coupled to Dacron grafts. As described in last year's report these grafts are thermally impregnated with heparin to minimize fibrinogenesis. The enzymatic properties of these grafts were examined in detail this year and it was found that the fixed enzyme has markedly altered Km's for each of its prototypical substrates - i.e. L-asparagine and L-glutamine, the amides; DONV (NSC-117,613) the diazo compound; and β-cyano-L-alanine, the nitrile. However the V max of the hydrolysis of L-glutamine by such grafts was actually increased so that the coupled enzyme was a faster L-glutaminase than its free counterpart. No hydrolysis of maleimide by the graft-bound enzyme was detectable even after prolonged incubations.

Moreover the graft bound enzyme could only briefly delete circulating L-asparagine of dogs into whose inferior vena cava it had been grafted. Thus the grafts as presently manufactured do not appear to have therapeutic potential.

14. Preliminary studies had shown that the antitumor proteins from Caesalpinia gilliesii (NSC-110,435) were able to release ammonia from the amino acids present in acid-hydrolyzed casein. (The other enzymatic properties of these not necessarily pure proteins are described elsewhere in this report). However, when the plant proteins were incubated with acid-hydrolyzed casein and the amino acid constitutents examined on the amino acid analyzer, no significant deletion of any amino acid was detected even after two hour incubation with high concentrations of the plant protein. Moreover in this experiment - which used boiled protein from Caesalpinia gilliesii as control, no significant ammoniagenesis could be detected.

Significance to Bio-medical Research and the Program of the Institute

The majority of the studies described above have focused on the synthesis or breakdown of L-asparagine. The studies on L-asparagine synthetase are directly relevant to the Chemotherapy program, NCI because the induction or function of that enzyme is the most likely explanation for the condition of resistance to the oncolytic enzyme L-asparaginase. If a powerful inhibitor of the synthetase can be found, it is conceivable that neoplasms not now amenable to enzymatic oncolysis, will be rendered susceptible. On the other hand if a powerful inhibitor of the synthetase does not confer sensitivity on L-asparaginase resistant tumors, the role of the synthetase in resistance to L-asparaginase will have to be reexamined.

Publications:

1. Morrison, R.K., Brown, D.E., Oleson, J.J., and Cooney, D.A.: Oral toxicology studies with Lapachol. Tox. & Applied Pharm. 17: 1-11, 1970.

2. Schaeppi, U., Thompson, T.R., Fleischman, R.W., Stadnicky, S., Phelan, R., Illievski, V., Cooney, D.A., Davis, R.D.: Phenester (NSC-116,785) Convulsant effects in the dog of an alkylating agent with antitumor activity. J. Tox. & Applied Pharm., in press, 1971.

3. Livingston, R.B., Venditti, J.M., Cooney, D.A., and Carter, S.K.: Glutamine antagonists in chemotherapy. Advan. Pharmacol. and Chemother. 8: 57-120, 1970.

4. Haskell, C.M., Canellos, G.P., Cooney, D.A., and Hansen, H.H.: Biochemical and pharmacologic effects of L-asparaginase in man. J. Lab. & Clin. Med. 75: 763-770, 1970.

5. Cooney, D., Davis, R., and Van Atta, G.: A spectrophotometric method for the simultaneous measurement of L-glutamine and L-asparagine in biological materials. Anal. Biochem., in press, 1971.

6. Cooney, D.A., Milman, H.A., and Truitt, R.: Disposable and versatile vessels for microdiffusion studies. Anal. Biochem., in press, 1971.

7. Rakieten, N., Gordon, B.S., Beaty, A., Cooney, D.A., Davis, R.D., and Schein, P.S.: Pancreatic islet cell tumors produced by the combined action of Streptozotocin and Nicotinamide. Proc. Soc. Exptl. Biol. Med., in press, May, 1971.

Serial No. NCI-6106
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: Effect of Antineoplastic Agents on Reproduction

Previous Serial Number: Same

Principal Investigator: Robert L. Dixon and Insu P. Lee

Other Investigators: Michael Innis

Cooperating Units: None

Man Years:

Total:	2 1/2
Professional:	1 1/2
Others:	1

Project Description:

Objectives:

IN VIVO

1. To study the effect of antineoplastic agents on male fertility using serial mating of mice.

2. To obtain fertility profiles by plotting percent fertile mice against days following drug treatment for various agents with known mechanisms of action.

3. To determine if the fertility pattern and spermatogenic cell type affected can indicate the mechanism of action of various drugs.

4. To evaluate the mutagenic potenital of anticancer agents.

IN VITRO

1. To describe the individual spermatogenic cell types which are capable of utilizing thymidine, uridine, and L-leucine as an indication of DNA, RNA, and protein synthesis.

2. To describe the effects of various antineoplastic agents on the cellular utilization of these substrates.

3. To study the duration of any drug-induced effects.

4. To correlate these observations with in vivo fertility studies on anti-neoplastic drug treated male mice.

Methods Employed:

IN VIVO

Serial mating of male mice treated with drugs was used to assess the biological functionality of sperm cells and to produce fertility patterns which have been shown to be inversely related in time to the phase of spermatogenesis damaged by the drug. For instance, drug effects on spermatozoa appear first, and those due to interference with spermatogonia appear last.

IN VITRO

The modified method of Lam, Furrer, and Bruce, which allows one to separate various spermatogenic cell types by velocity sedimentation is being used. Testicular cells are prepared by removing the tunica albuginea from the testes under a stereomicroscope, and cutting the testicular tissue into short segments. This tissue is washed into a Nalgene tube with 10 mls of Spinner solution containing 0.5% bovine albumin. Subsequently, the cells are suspended by gently pipetting the suspension up and down with a Nalgene pasteur pipette. Following this, the heavy tissue segments are allowed time to settle. The supernatant of the cell suspension is filtered through a lens paper to remove cell aggregates. Cell number is then determined with cell counter. 2×10^7 cells are loaded into the sample vessel and 720 mls of a 1-3% linear gradient of bovine serum albumin in Spinner solution is developed through the bottom of the chamber. At the end of 4 hours of sedimentation, the chamber is drained from the bottom at the rate of 10 mls per minute. 135 individual fractions are collected with an LKB fraction collector. Each fraction corresponds to a sedimentation distance of 0.18 mm. The chamber size is such that 1 mm of vertical distance contains 7.2 mls of gradient fluid.

Following cell fractionation, each sample is filtered and the cells collected on a Millipore Filter (0.5 μ pore size), and the acid insoluble fraction of nucleic acids and proteins are obtained. Each fraction is dried under a hood and then digested with 0.5 mls of solubilizer at 50°C in a mechanical convection oven for two hours. After cooling the vials, 10 mls of toluene containing Liquiflor is added to each vial. The radioactivity in the acid insoluble portion of each fraction is then determined.

To determine the volume of individual cells, an Intertechnique Didac multi-channel analyzer was interphased with a Celloscope which analyzed the pulse height signal for cell volume.

The morphology of the cells in each fraction was examined by the exfoliative cytological technique which also allows the cells on each slide to be counted. An Ultraphot II Zeiss camera microscope was used to prepare photomicrographs of individual slides.

Major Findings:

IN VIVO

In vivo studies of the fertility of male mice have continued during the past year. However, the findings are essentially those reported in last year's report (with the exception of L-asparaginase) and can be summarized as follows:

1. Saline Treated Controls. Saline pretreated male mice demonstrated a fertility rate of 80-100% following the second week of serial mating. It appears that two 5-day breeding periods are necessary for the male to acclimate to the new environment. The average control litter size was 9.5 with a standard deviation of 1.9.

2. Cytosine Arabinoside. Cytosine arabinoside, an inhibitor of DNA synthesis, was most effective when administered every three hours for 24 hours. Normal reproduction was observed initially, and a decrease in fertility followed more than four weeks after treatment.

3. Methotrexate. The results with methotrexate were essentially the same as those seen with cytosine arabinoside.

4. Cyclophosphamide. Cyclophosphamide was most effective after a single treatment as were the rest of the drugs to be presented. This drug was capable of affecting non-proliferating cells (spermatids) and also had an effect, although less dramatic, on early stages of spermatogenesis involving DNA synthesis.

5. Vincristine. Vincristine is thought to cause an arrest of cell division. This drug was effective against all spermatogenic cell types. The antifertility mechanism may include not only an arrest of cell division, but also a decreased motility of the developing sperm (spermatids).

6. Procarbazine. Procarbazine had the most potent antifertility effect. This drug effected all cell types and the effect appears to be only slowly reversible. This fact indicates that a large number of the spermatogonic stem cells might have been destroyed by drug treatment.

7. L-Asparaginase. L-Asparaginase had a significant effect on male fertility throughout a forty-day test period. In addition, this oncolytic enzyme produced a significant number of reabsorbed fetuses.

IN VITRO

The major portion of this year's research was related to spermatogenic cell separation and a study of the effect of antineoplastic drugs on the incorporation of thymidine, uridine, and L-leucine by various individual spermatogenic cell types. The cellular incorporation of radioactive thymidine,

uridine or L-leucine was studied one hour after intraperitoneal injection of the nucleoside or amino acid. The thymidine peak fraction was identified as spermatogonia which had a sedimentation rate of 5.5 mm/hour. Tritium labelled uridine appeared in the cell fraction sedimenting at 3.9 mm/hour when cells are isolated one hour after injection of the substrate. This peak of activity represents intermediate spermatids. The cell types that incorporated tritium labelled L-leucine one hour after intraperitoneal administration of the amino acid were also identified: a 1.1 mm/hour fraction contained late stages of spermatids and some spermatozoa; a 5.5 mm/hour fraction contained spermatogonial cells; a 8.7 mm/hour fraction represented early spermatids; a 13.1 mm/hour fraction were secondary spermatocytes, and a 17.8 mm/hour fraction was identified as diplotene and pachytene cells.

Five antineoplastic agents were tested for their effect on the incorporation of thymidine, uridine, and L-leucine. Cytosine arabinoside, vincristine, cytoxan, L-asparaginase, and procarbazine were administered intraperitoneally at the maximally tolerated dose. The cell types followed were the spermatogonia, intermediate spermatids, and the late spermatids. Groups of treated mice were sacrificed one hour after drug treatment, and also at 5, 10, 15, 20, and 30 days after drug injection. In every group, the thymidine, uridine, or L-leucine was injected one hour prior to sacrifice.

The following is a summary of the results obtained when the nucleosides or the amino acid were administered one hour after drug treatment:

1. Cytosine arabinoside effect was predominantly on the spermatogonial cells (thymidine incorporation) reflecting an effect of DNA synthesis.

2. Cytoxan affected both thymidine and uridine utilization.

3. Vincristine had its most significant effect on uridine incorporation and protein synthesis.

4. The major action of Procarbazine appeared to be on thymidine and uridine incorporation.

5. The predominant effect of L-asparaginase was on uridine incorporation and protein synthesis.

Significance to Bio-Medical Research and the Program of the Institute:

Studies of the effects of antineoplastic agents on reproduction in the past have centered largely on teratogenicity. Few investigations have been concerned with the effect of these drugs upon fertility. However, as more effective drugs and therapeutic regimens have been developed, questions concerning the effect of anticancer agents on reproduction are becoming clinically important.

Van Thiel and DeVita have recently documented the pronounced effect of an antineoplastic drug combination on male spermatogenesis. Seven patients in

remission after receiving chemotherapy for lymphoma were evaluated 1-5 years after treatment. Persistent azoospermia was present in 6 of 7 men. Testicular biopsy in these azoospermic men showed complete absence of all germinal elements. However, Sertoli cells and Leydig cells were present.

In addition to the clinical aspects, spermatogenesis appears to present an interesting population of cell types for basic studies dealing with drugs that effect cell multiplication. Spermatogonial cells mature in a highly synchronous fashion into only one type of differentiated cell, the spermatozoan. It also should be pointed out that the spermatogenic cell population provides an excellent model system to study the effect of drugs on cells that are not dividing (spermatids, spermatozoan). Spermatogenesis might be a valuable test system to screen for drugs specifically effective against nonproliferating cell populations.

Planned Course:

These studies will be continued and expanded to assess carefully the effects of antineoplastic agents on reproduction. Attempts will be made to correlate these data with clinical findings. Future investigations will include an evaluation of the mutagenic potential of oncolytic agents using the "dominant lethal" technique, and also a study of the effect of these chemotherapeutic drugs on ovarian function. In addition, the value of rodent spermatogenesis as a means of testing for drugs effective against nonproliferating cell populations will be further investigated.

Serial No. NCI-6107
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Program
July 1, 1970 through June 30, 1971

Project Title: Biochemical Pharmacology of Antineoplastic Agents

Previous Serial Number: Same

Principal Investigator: Aron Primack

Other Investigators: Robert L. Dixon

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	1
Others:	1/2

Project Description:

During the past year, studies of the metabolism and toxicity of cyclophosphamide have been initiated. Cyclophosphamide is an important antineoplastic agent in wide clinical use. The drug is administered in an inactive form and requires enzymatic conversion to an active metabolite. This metabolic activation of cyclophosphamide varies between animal species and within the same species after treatment with a variety of other drugs. To date the active metabolite has neither been isolated, identified, nor tested for antitumor efficacy. These experiments have been designed to approach this problem using recently reported column chromatographic procedures to separate the metabolites of cyclophosphamide.

The usual toxicities associated with cyclophosphamide are white blood cell depression and cystitis. Bladder irrigation with sulfhydryl containing compounds has been tested in an attempt to control the cyclophosphamide-induced chemical cystitis.

Objectives:

1. To determine the metabolites of cyclophosphamide after *in vitro* activation and after administration of the drug to animals and man.

2. To assess the antibacterial effect of the urinary metabolites of cyclophosphamide.

3. To evaluate the antitumor activity of various cyclophosphamide metabolites.

4. To study the interaction of cyclophosphamide and potassium iodide.

5. To investigate the effect of acetylcysteine on cyclophosphamide-induced cystitis in dogs and monkeys.

Major Findings:

A number of cyclophosphamide metabolites have been successfully isolated using L-20 Sephadex chromatography. Studies have been conducted using dog, monkey, and man. Each species appears to produce a unique chromatographic profile of metabolites which indicates variations in metabolism. These data have been compared with the relative toxicity of cyclophosphamide in the individual species to determine if these metabolic patterns correlate with toxicity, and therefore can explain some of the biological variation seen with this drug. In addition, *in vitro* acid hydrolysis of cyclophosphamide produces a large variety of NBP positive (test for alkylating agents) metabolites.

Following a clinical report of apparent antibacterial activity of the urinary metabolites of cyclophosphamide, metabolites produced both *in vitro* and *in vivo* were tested for their effect on a variety of clinically important bacteria. In no case was there significant antibacterial action associated with the metabolites of cyclophosphamide.

Studies are underway to assess the antitumor potential of various metabolites of cyclophosphamide using L-1210 leukemia. Initial results indicate that toxicity and antitumor activity of the various metabolites vary.

The interaction of cyclophosphamide and potassium iodide (KI) was studied under various conditions following a clinical report that KI increased the effectiveness of cyclophosphamide. It was demonstrated that potassium iodide increased the toxicity of the drug, but was ineffective in prolonging the survival time of cyclophosphamide-treated L-1210 leukemia mice. Potassium iodide had no effect on hexobarbital sleeping times which is an *in vivo* indicator of drug metabolizing enzyme activity. The mechanism of the KI potentiation of cyclophosphamide lethality is undetermined.

The effect of acetylcysteine on cyclophosphamide-induced cystitis in dogs and monkeys was studied. Acetylcysteine, administered by bladder irrigation, was demonstrated to effectively ameliorate the drug-induced cystitis. In addition, it did not appear the acetylcysteine altered the systemic effect of the alkylating agent in spite of apparent absorption of the sulfhydryl agent from the bladder. Further studies in the Beagle are in progress to assess the effect of concomitant oral and/or parenteral administration of acetylcysteine and cyclophosphamide. These results will form the basis for future clinical studies to test the value of bladder irrigation in the control of cyclophosphamide-induced cystitis.

Significance to Biomedical Research and the Program of the Institute:

Cyclophosphamide is an effective, clinically useful, antineoplastic agent.

It is a unique drug in that it requires enzymatic conversion to an active metabolite. High doses of the drug are often used and can result in cystitis being the dose-limiting toxicity. Further information regarding metabolism, active metabolites, and methods to ameliorate the drug-induced toxicity are of obvious clinical value. These studies will not only aid in the understanding of the activation of cyclophosphamide, and perhaps increase the therapeutic index of cyclophosphamide; but might also result in newer and even more effective agents.

Future Work:

This area of research will be continued within the Laboratory of Toxicology during the coming year. In addition, collaborative studies will be conducted with Dr. Primack who will be at the Lymphoma Treatment Center, Kampala, Uganda, Africa next year.

Publications:

1. Primack, A.: The interaction of cyclophsophamide and potassium iodide. Proc. Soc. Exp. Biol. & Med., In Press, 1971.

2. Primack, A. and Dixon, R.L.: Amelioration of cyclophosphamide-induced cystitis. JNCI, In Press, 1971.

3. Primack, A., Wilson, J., O'Conor, G., Engelman, K., and Canellos, G.P.: Hepatocellular carcinoma with the carcinoid syndrome. Cancer, In Press, 1971.

4. Scholl, G., and Primack, A.: Uptake of ^{85}Sr by osteosarcoma metastases to lung. J. Nuclear Medicine 12: 131-133, 1971.

Serial No. NCI-6110 (new)
1. Laboratory of Toxicology
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1970 through June 30, 1971

Project Title: The Pathology of Drug-Induced Alterations in Animals and Man

Previous Serial Number: None

Principal Investigator: David M. Young

Other Investigators: Gerhard R. Krueger

Cooperating Units: Laboratory of Pathology, General Laboratories and Clinics, National Cancer Institute

Man Years:

Total:	1
Professional:	1/2
Other:	1/2

Objectives:

The basic objectives of this project are (1) to contribute to a further understanding of the biogenesis of drug-induced lesions utilizing biochemical, cytochemical and morphological methods; (2) to evaluate the predictability of preclinical toxicology by comparing drug-induced toxicity in test animals with that occurring in man; and (3) to define those drug-induced diseases (syndromes) in test animals which may serve as models for study of spontaneous disorders.

Proposed Study:

Anticancer Drug-Induced Disease. Nearly all drugs produce evident side effects but few have the potential to induce such dramatic changes in body chemistry and tissues as the anticancer agents. The majority of these agents are, in fact, tissue poisons. A thorough investigation into the pathogenesis of anticancer drug-induced alterations would result in the following beneficial effects in two areas of research: 1) applied - with the elucidation of the mechanism of drug-induced toxicity, one could then attempt to nullify the toxicity while retaining the full oncolytic capability; 2) basic - with the definition of pathologic alterations produced by drug administration, one could establish an experimental model to study spontaneous diseases of animals and man. Investigations of the effects of Streptozotocin (NSC-85998) have emphasized the benefits of this biphasic approach. Nicotinamide has been shown to ameliorate the diabetogenic effects of this agent in animals

without a resultant loss of antitumor activity. In addition, Streptozotocin-induced diabetes appears to have several advantages over the alloxan diabetic animal model currently used. In order to destroy the β-cells with alloxan, such large doses are required that fatal hepatotoxicity and renal toxicity may develop. In addition alloxan administration results initially in triphasic changes in blood glucose levels. Another model involves Streptozotocin-induced renal changes of glycogen deposition in proximal convoluted tubules with accompanying glycosuria, ketonuria, aminoaciduria, and acidosis. Many of these changes are comparable to those seen in hereditary renal disease (Fanconi syndrome). Therefore, this drug-induced nephropathy may serve as a model system for the study of hereditary functional abnormalities of the kidney.

The problems involved in attempting to associate specific lesions with a particular drug admist the shower of polypharmacy besieging the average clinical cancer patient is fully appreciated. Therefore, initial studies will concern only the following three parent drugs as they appear to leave a specific "fingerprint" toxicity:

(1) Bleomycin (NSC-125,066) - integumental-pulmonary toxicity.

(2) Daunomycin (NSC-82151) and/or Adriamycin (NSC-123,127) - cardiovascular toxicity.

(3) Nitrosourea agents (NSC-409,962, NSC-79037, NSC-95441) - delayed bone marrow depression.

These preliminary investigations involve a collaborative effort between the Laboratories of Toxicology and Pathology, NCI, NIH and the Tissue Reactions to Drugs Registry, Armed Forces Institute of Pathology (Dr. Nelson S. Irey, Registrar).

In order to fulfill the objectives of this study, the principal investigator currently attends autopsy review sessions on cancer patients (NIH Clinical Center), obtaining tissues for evaluation when necessary. These findings are correlated with those reported by contractors in preclinical studies in dogs and monkeys.

Other Projects:

1. The Effects of Streptozotocin on Parathyroid Hormone and Calcitonin. Previous studies have suggested that Streptozotocin-induced hypoglycemia is the result of degranulation of beta cells in the islets of Langerhans. Perhaps the administration of Streptozotocin results in degranulation of other polypeptide hormone producing cells. The development of a definite hypocalcemia and hypophosphatemia with eventual metabolic bone changes suggests an alteration in the dual hormonal control of the calcium homeostatic mechanism (parathyroid hormone and calcitonin). The present study is undertaken to test the hypothesis that altered calcium homeostasis occurs in Streptozotocin-treated animals.

2. The Pathogenesis of L-asparaginase-Induced Hypocalcemic Tetany in Rabbits. Recent reports suggest that the administration of L-asparaginase to rabbits results in selective necrosis of the parathyroid glands with subsequent development of hypoparathyroidism, hypocalcemia, tetany and death. Further investigations are required to elucidate the mechanism of this most interesting selective toxicity.

3. The Interrelationships of Calcitonin and Histaminase in Medullary Carcinoma of the Thyroid Gland. Previous investigations at NIH have revealed elevated levels of histaminase activity in both serum and tissue specimens from patients with medullary carcinoma. This neoplasm is derived from the calcitonin-producing parafollicular (C-cells) of the thyroid gland. Appearance of abnormal histaminase levels is usually indicative of metastatic disease. Therefore, following the serum histaminase activity in patients may provide a means for following the course and effects of chemotherapy. A further understanding of the biochemical pathology of medullary carcinoma of the thyroid gland is required in order to improve the chemotherapeutic effectiveness in this disorder. These investigations are performed in collaboration with Dr. Stephen B. Baylin, NHLI.

4. The Effects of Cancer Chemotherapeutic Agents on Lymphoreticular (Immunogenic) Tissues. Nearly all antineoplastic agents have the capability of immunosuppression. Current studies are being undertaken to determine the effects of these immunosuppressive drugs on the morphology of lymphoreticular tissues. This study is being performed on a comparative basis with histopathologic evaluation of tissues from dogs, monkeys and man. Dr. G. R. Krueger is collaborating in this effort.

5. Toxicity of Hormones. A large number of endocrine compounds have been screened for anticancer activity. Hormone therapy of cancer has developed during the past 25 years with notable advances in management of breast carcinoma, carcinoma of the prostate and endometrial carcinoma. Since the majority of the endocrine active agents resemble the structure of endogenous endocrines, the toxicity is usually manifested by an exaggerated target-organ response. Current preclinical toxicological studies involve Estriol (NSC-12619E), Estradiol Mustard (NSC-112,259E) and Phenesterin (NSC-104,496E).

6. Pathologic Evaluation of Spontaneous Leukemia in AKR Mice. Recent studies have suggested that the spontaneous lymphoma in AKR mice may be an excellent experimental model for screening of cancer chemotherapeutic agents. In order to evaluate the effects of drugs on this neoplastic process, an initial investigation of the variations of the spontaneous disorder must be undertaken. Once the baseline tumor involvement has been established, studies involving the effects of drugs on regression of development will be initiated. Evaluation will include thorough necropsies with gross photography of significant lesions, histopathologic observation and photomicrography of representative areas. These studies are performed in collaboration with Dr. John P. Glynn, CCNSC, NCI.

7. The Role of Altered Mineral Metabolism in Daunomycin-Induced Cardiotoxicity. Previous studies have reported ultrastructural changes in cardiac tissue from Daunomycin treated mice. Mitochondria appeared to have fewer electron dense granules and sarcomeres contained disorderly, nonparallel arrays of myofibrils. The present study is undertaken to determine the role of divalent cations (e.g. Ca^{++}, Mg^{++}) in this myocardial alteration. Perhaps precipitates of mineral salts either intramitochondrially or upon myofibrils could result in altered membrane permeability and interruption of myocardial conductance. Similar changes have been reported occasionally in patients with congestive heart failure. Therefore, this study is undertaken to determine the role of these alterations in the pathogenesis of Daunomycin-induced cardiotoxicity.

Significance to Bio-Medical Research and the Program of the Institute:

The need for a better understanding of the pathologic effects of anticancer agents cannot be overemphasized. Upon elucidation of the pathogenesis of drug toxicity, methods can be explored to nullify the adverse effects while retaining the drug's anticancer potential. A by-product of these studies of drug-induced lesions is the possible definition of new experimental models to study spontaneous diseases in animals and man. For example, Bleomycin-induced toxicity may be an excellent model to study connective tissue diseases. A thorough study of drug-induced lesions on a comparative basis (dog, monkey and man) will yield valuable information on the predictiveness of the current preclinical toxicology protocol.

Investigations of the effects of cancer chemotherapeutic agents on spontaneous tumors in mice may result in the definition of a model system more nearly comparable to the natural occurring disease in man. Studies of the progression of neoplastic disease in the face of drug administration will contribute to a further understanding of use of model systems for cancer chemotherapy.

Publications:

1. Young, D.M. and Capen, C.C.: Thyrocalcitonin: Response to experimental hypercalcemia induced by vitamin D in cows. In S. Taylor (Ed.): Calcitonin 1969: Proceedings of the Second International Symposium. London, Wm. Heinemann Medical Books, Ltd., 1970, pp. 141-153.

2. Capen, C.C. and Young, D.M.: Ultrastructural evaluation of the secretory activity of the bovine parathyroid glands and thyroid parafollicular cells under normal, pathologic and experimental conditions. In J.J.B. Anderson (Ed.): Parturient Hypocalcemia. New York, Academic Press, 1970, pp. 207-229.

3. Rowland, G.N., Capen, C.C., Black, H.E., and Young, D.M.: Bone turnover studies associated with experimental alterations of calcium metabolism in cows. Calcif. Tissue Res., in press, 1970.

4. Young, D.M. and Capen, C.C.: Thyrocalcitonin content of ultimobranchial tumors from bulls. Path. Vet., in press, 1970.

NSC-742 Azaserine Mason Research Institute, Inc.

In an acid environment Azaserine is degraded to gaseous nitrogen and uncharacterized products. The degraded compound, without an attached diazo group, is nontoxic by oral and intravenous administration. Because of the known acid-catalyzed hydrolysis of Azaserine, it was anticipated that orally administered drug would not survive the gastric acidity of the canine stomach. However, oral administration was equi-toxic with the intravenous route so that a major portion of the drug, or a toxic product therefrom must have been absorbed. The salient toxicity of Azaserine is the production of pathologic alterations in canine submandibular glands. This is explicable by the finding that salivary-gland homogenates are extremely active in decomposing Azaserine. Since this agent was very promptly toxic to the lymphocytes it may be that these cells are decomposing the drug to diazoacetate by the same mechanism. It is more likely, however, that intact Azaserine is antagonizing the utilization of L-glutamine for nucleic acid synthesis in lymphocytes, although both modes of action (glutamine-antagonism and alkylation) could operate coincidently. When Azaserine was degraded by acid in vitro and the degradation products given orally or intravenously, no drug-related toxicity was seen.

NSC-12169E Estra-1,3,5(10)-triene-3,16α,-17β-triol

Estriol

Mason Research Institute, Inc.

Estriol, a metabolite of estradiol and estrone, had rather mild toxic manifestations when administered orally to the dog and monkey. The major alterations were edema of vulvar, scrotal and perianal regions. Although abnormal liver function parameters were noted in one monkey, these were not thought to be drug related as they did not occur at higher doses in either species. Squamous metaplasia, atrophy of prostate and vacuolar degeneration and atrophy of the testes and epididymides were the sole pathologic changes noted.

NSC-18429 Aniline, N,N-bis (2-chloroethyl)-

Mason Research Institute, Inc.

The toxicity of Aniline mustard was studied in Rhesus monkeys at doses ranging from 12.5 mg/kg to 100 mg/kg administered orally once a week for a period of eight weeks. Although erythropoiesis was depressed significantly at all doses, the changes were reversible. Leukopenia, mainly neutropenic, was similarly noted at all doses although the severity appeared to be dose-related. Moderate thrombocytopenia appeared only at the higher two doses studied. The administration of Analine mustard produced persistent steatosis of myocardium, liver, and kidney. Histopathologic evaluation of tissues from control monkeys did not reveal these alterations. No clinical signs of altered myocardial

function were seen, but a systematic study of this point was not undertaken. Several animals at higher doses exhibited early and delayed sporadic retention of BSP which was the only altered chemical parameter suggestive of hepatic steatosis. A weekly dosage schedule did not moderate the myelotoxic potential of Aniline mustard.

NSC-52695 Phosphorodiamidic acid, N,N-bis(2-chloroethyl)-N'-(3-hydroxypropyl)-, compd. with cyclohexylamine (1:1) — Charles Pfizer and Company

Cytoxyl alcohol

The toxicity of cytoxyl alcohol was evaluated in Beagle dogs following intravenous administration of single doses, two doses a week apart, and five daily doses. Toxic signs were qualitatively similar for all regimens. The toxic manifestations concerned primarily the urinary system and appeared to reflect a direct and prolonged cytotoxic effect on the tissues and organs of this system. The severity and rate of onset of toxic signs were dose related. However, some irreversible changes (BUN, PSP) were not apparent until weeks after treatment. Polyuria, polydipsia, hyposthenuria, proteinuria, and hematuria were prominent at all toxic doses and persisted at high doses. BUN elevation and reduced PSP excretion, usually delayed in onset, occurred also at high doses; reduced PSP excretion persisted for as long as 81 days. Other signs of toxicity, i.e. leukocyturia and hyponatremia, were not consistent findings. The most prominent anatomical changes seen involved the urinary tract. These alterations ranged from mild cystitis to thickening, hemorrhage, ulceration, and necrosis of the bladder. Additional histopathologic alterations included loss of bladder epithelium, edema, necrosis and cellular infiltration of the lamina propria with deposition of fibrin. Gross renal pathologic changes included cortico-medullary hemorrhage and enlargement of the pelves; microscopic alterations were characterized by focal hemorrhage, infiltration and tubular dilation. The early onset of hyposthenuria and hponatremia indicated renal toxicity independent of the subsequent hydronephrotic changes.

Rectal bleeding and bloody feces were characteristic findings indicative of gastrointestinal toxicity. Diarrhea, incontinence, and mucosal hyperemia were also seen inconsistently. There were no significant histopathologic gastrointestinal findings.

Cardiovascular toxicity was indicated by consistent electrocardiographic changes which included increased amplitude and inversion of the T-wave (Lead II and Lead CV6LL). These alterations were reversible.

Neuromuscular toxicity was indicated by hypoactivity, posterior weakness and ataxia seen at the higher doses. In view of the delayed expression of toxicity and the extraordinary potential for irreversible damage to the bladder and kidneys, cytoxyl alcohol was not recommended for clinical use.

NSC-56654 Azotomycin Charles Pfizer and Company

The present studies were designed to investigate the influence of dosage schedule upon the toxicity induced by Azotomycin. Three schedules were used; a single rapid injection, a single twenty-four hour infusion, and weekly doses for six weeks. The study confirmed previous observations that the drug is best tolerated by rapid intravenous administration. With all dosage regimens, however, the parameters of toxicity were similar. Emesis and anorexia were noted at all doses tested. Bloody feces and pathologic changes of gastrointestinal epithelium were seen at higher doses. Large doses of Azotomycin destroyed numerous gastrointestinal cells regardless of dosage regimen. Adequate numbers of cells for repopulation remained after single administration; whereas, prolonged exposure by infusion or repeated doses every seven days depleted all potential regenerators with subsequent death of the animal. Hemoconcentration was commonly observed. As doses were increased, effects on bone marrow and lymphoid system were indicated by a fall in the total number of granulocytes, lymphocytes and platelets. Hepatotoxicity was indicated by increased transaminase and alkaline phosphatase levels. Increased BUN levels, proteinuria, polydipsia and polyuria were suggestive of nephrotoxicity. Heart and lung toxicity was indicated by epicardial and endocardial hemorrhages and focal pulmonary fibrosis with mild chronic pneumonitis. At high doses, electrocardiographic changes were recorded. A dose of 100 mg/kg was fairly well tolerated as a single injection but when given as a 24 hour infusion, the toxicity rose significantly. Part of this increased toxicity may stem from the fact that the drug was delivered directly into the right atrium and that the drug appeared to pass easily through the endocardium into the myocardium.

NSC-65346 Sangivamycin Charles Pfizer and Company

Since Sangivamycin was found to be cardiotoxic in the clinic, the agent was restudied in several lower species with the hope of determining if the toxicity might be predictable and manageable.

In the anesthetized dog and cat reversible hypotension followed the intravenous administration of Sangivamycin at doses ranging from 0.5 mg to 12 mg/kg. Apart from one burst of extrasystoles immediately after dosing, there was little electrocardiographic evidence of toxicity. In the small number of heart tissue samples submitted for histopathologic evaluation, no damage to the myocardium was detected. Young beagles recieved two courses of the drug at doses of 0.5 to 1.0 mg/kg for three to five days. The first course was tolerated without signs of obvious cardiac toxicity but with marked hepatotoxicity. During the second course, 4 to 33 days later, mild systolic murmurs were detected in the puppies along with electrocardiographic abnormalities which consisted of variations of the PR interval and A-V dissociation with an ectopic pacemaker. It was not possible to produce electrocardiographic or ausculatory changes without prior or concommitant laboratory or clinical evidence of liver involvement. In this study Sangivamycin was again shown to be toxic to the hepatobiliary apparatus.

The administration of the drug induced severe hemorrhagic necrosis of the liver and inflammation and/or necrosis of the gall bladder. In the treated puppies, striking atrophy of the thymus was detected. Thus, Sangivamycin appears to be lymphotoxic to these immunologically important cells. The effect of the depletion of thymocytes on the immune system was not, however, determined.

NSC-82196	Imidazole-4(or 5)-carboxamide, 5(or 4)-[3,3-bis(2-chloroethyl)-1-triazeno]-	South Shore Analytical & Research Lab.,Inc.

In this study freshly prepared drug was injected intravenously in monkeys at doses of 30 and 60 mg/kg every fourth day to a total dose of 660 mg/kg. The sole pathologic effect of drug administration was a gradually developing reversible mild to marked leukopenia. Because of volumetric limitations, no higher doses were studied and therefore a lethal dose was not achieved.

NSC-87974	Urea, 3-cyclohexyl-1-(2-fluoroethyl)-1-nitroso CFNU	Mason Research Institute, Inc.

CFNU was given orally to Rhesus monkeys in large single or small repeated doses for the purpose of exploring its chronic effects. Hepatic cirrhosis was specifically anticipated and was demonstrated. No fibrosis was detected. However, not only the liver but also the heart was significantly infiltrated with fat. In addition, focal moderate chronic inflammation of the heart, kidney and liver was detected in one or both of the paired monkeys used for each of the dosage schedules studied. A sporadic spike of one or both transaminases or a mild reversible retention of BSP might be correlated with these fatty or inflammatory changes. The toxic effects of this nitrosourea were long-lasting and difficult to predict.

NSC-102,627	1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride	South Shore Analytical & Research Lab.,Inc.

In dogs, the principal targets of toxicity were bone marrow and liver. Reversible leukopenia was the chief manifestation of the myelotoxicity of this agent. However, mild to moderate anemia and thrombocytopenia sometimes accompanied the depression of leukocytes. After single doses of drug, the nadir of leukopenia was reached on day 8 with evidence of recovery by day 10. When a second identical dose of drug was given at the nadir of leukopenia, no evidence of increased myelotoxicity was seen. After one dose, the recovery time of bone marrow from the nadir of leukopenia was approximately 2 days; after 5 doses this recovery time was approximately 15 days; and on the 5-9-5 dosage schedule, it was approximately 23 days. Therefore, although these data were derived from only one or two animals, it appeared that

repeated insults with the drug left the marrow less able to recover quickly. Chemical, but not morphologic evidence of hepatotoxicity was seen after the administration of severely toxic single or repeated doses of the agent. Cholecystitis was a sporadic finding in the multiple-dosed beagles. NSC-102,627 was toxic to the gastrointestinal system in mice; diarrhea and bloody intestinal contents were the chief manifestations of this toxicity. No gastrointestinal toxicity was observed in the dogs. On the basis of the mouse lethality data it appeared that the toxicity was not cumulative. However, in both the mouse and dog, the dose-response curve was extremely steep. In the female mouse given five-daily doses, the LD_{10} was 73 mg/kg, the LD_{50} 81 mg/kg and the LD_{90} 88 mg/kg. Similarly in the dog 5 mg/kg/day x 5 was approximately the LD_{50}; half of this dose was approximately the maximum tolerated dose.

NSC-102,816 s-Triazin-2(1H)-one, 4-amino-1-β-D-ribofuranosyl- A. D. Little, Inc.

5-Azacytidine

5-Azacytidine, a compound synthesized and studied thoroughly in the Czechoslovakian SSR, was selected for toxicological evaluation on the basis of its oncolytic activity against mouse L1210 and several tumors of man.

The toxicologic effects of the compound can be grouped into two areas: toxic effects on selected rapidly dividing cells, and toxic effects on interphase cells of important parenchymal organs.

5-Azacytidine proved to be only a moderate panmyelotoxin even at the highest single dose studied. The level of circulating leukocytes was not obliterated by the drug. Thrombocytes appeared to be the least and most slowly susceptible elements of the marrow. In the wake of myelosuppression, secondary inflammation and infestation of the lungs appeared to supervene. The inflammation resembled distemper and could corroborate the observation of Zendzian *et al* that MTX unleashes canine distemper virus in treated dogs.

Only the very highest doses of 5-azacytidine studied brought about widespread necrosis of lymphatic organs. However, at 13 mg/kg x 1, the karyorrhexis of the thymus and nodes was dramatic.

In contrast to the drug's panmyelotoxicity, 5-azacytidine was without detrimental effect on the epithelium of the gastrointestinal tract.

Moderate single and repeated doses of 5-azacytidine were damaging to the liver. Elevations of SGPT and alkaline phosphatase but not ordinarily of the SGOT pointed to this effect as did prolonged prothombin time. Fatty or necrotic changes were detected histopathologically in the hepatocytes. The toxicity of 5-azacytidine appeared to be predictable dose-related and reversible in most instances with hepatocyte necrosis being the exception. This toxic effect to the liver was thought to be a reflection of interference by 5-azacytidine with the active pyrimidine metabolism of the liver.

While the principal morphologic effects of 5-azacytidine on the kidney were not remarkable, significant elevations of BUN were consistently seen in animals receiving toxic doses of the drug. However, on the basis of their fluid intakes, most of these animals were not considered dehydrated although elevations of hematocrit did tend to accompany elevations of BUN.

NSC-109,229 L-Asparaginase Charles Pfizer & Company
NSC-56654 Azotomycin

Since Azotomycin is being used in combination with L-asparaginase in the treatment of human leukemias, and because Azotomycin given with L-asparaginase has been found to produce a synergistic effect against the L5178Y tumor in mice, acute toxicologic studies were undertaken to explore the interaction of these agents in normal mice.

The single dose LD_{50} of Azotomycin was found to be approximately 250 mg/kg; while the five day LD_{50} was approximately 0.7 mg/kg. Thus, it has been confirmed that the toxicity of the drug rises sharply when dosage is repeated.

L-Asparaginase did not increase the toxicity of single doses of Azotomycin but appeared to augment the toxicity of repeated doses. These results are similar to those obtained in the L5178Y tumor system where it was found that toxicity of two repeated doses of Azotomycin (days 1 and 5) plus L-asparaginase was markedly increased once a dose of 5 mg/kg was exceeded. The therapeutic synergism between L-asparaginase and Azotomycin was prominent at lower doses whereas the toxic synergism became prominent only at high doses.

The concentration of L-glutamine in the livers of animals receiving one dose of 600 mg/kg of Azotomycin by the intraperitoneal route rose markedly on the day after dosing whereas the concentration of L-asparagine was unchanged. The elevation of hepatic L-glutamine was maintained for at least 72 hours after this large dose in spite of the fact that the liver weight fell significantly. Evidence was obtained that Azotomycin was inhibiting the reactions utilizing L-glutamine rather that the synthesis of L-glutamine. Although Azotomycin is a strong inhibitor of L-asparagine synthetase, its failure to lower the hepatic levels of this amide appeared to indicate either that such inhibition was transitory, or that dietary L-asparagine could supply the requisite intracellular concentrations.

Azotomycin did not appear to produce significant gastrointestinal lesions in mice although some diarrhea was seen when the drug was combined with L-asparaginase. It will be recalled that gastrointestinal toxicity is prominent in larger animals and man receiving the drug.

NSC-110,435 Cesalin (Protein, from *Caesalpinia gilliesii*) Mason Research Institute, Inc.

Cesalin is an oncolytic plant protein active at very low doses against the

Walker carcinosarcoma of rats. In vitro studies were designed to study the hypothesis which proposed that this protein is in fact an enzyme. No lipase, phosphatase amylase, hyaluronidase, glucose oxidase, hexokinase, lactate dehydrogenase, G-6-P-dehydrogenase, carbonic anhydrase, protease, deoxyribonuclease or alkaline phosphatase activities were discovered. Cesalin did, however, exhibit a feeble ribonuclease activity near neutral pH; a modest acid phosphatase action, slight butyrylcholinesterase activity, and considerable ability to use DPNH and TPNH as cofactors for the dehydrogenation of some substrates present in perchloric acid extracts of liver. It is impossible to say which of these activities, if any, was related to the oncolytic properties of this plant protein. Cesalin deaminated one or more of the amino acids contained in acid-hydrolyzed casein. These findings are being further explored.

NSC-111.071 Carbenicillin disodium (USAN) — South Shore Analytical & Research Lab.,Inc.

Carbenicillin was administered intravenously to Beagles alone or simultaneously with Gentamicin. The dosage regimens used were: 1) Carbenicillin, 200 mg/kg every four hours for seven days or 2) Carbenicillin, 200 mg/kg, plus Gentamicin, 0.6 mg/kg every four hours for seven consecutive days. Neither dosage regimen produced any overt toxic signs in the animals, nor any significant changes in the hematology, blood chemistry, urinalysis; nor any gross or histopathologic alterations in the animals apart from phlebitic problems with the indwelling catheters. During the course of study, the leukocyte counts were decreased marginally in the animals treated with both drugs. However, these changes were not reflected in any signs of toxicity or evidence of pathologic changes.

NSC-116,785 Acetic acid, [p-[bis(2-chloroethyl) amino]phenyl]-, ethyl ester

Phenester — Mason Research Institute, Inc.

Oral administration of Phenester caused very severe convulsions in the Beagle at all doses above the clinically tolerated dose of 3.5 mg/kg/day x 5 days. In addition, severe erratic hypoplasia of the marrow and lymphoid tissue was seen at all doses above 3.5 mg/kg/day x 5 days. In addition, it appeared that animals intoxicated with Phenester might be unusually susceptible to bacterial infections. At all doses, including the clinically tolerated dose, inflammation and fatty changes of the liver and myocardium were observed. Incomplete drug absorption and vomiting probably accounted for the poor dose response relationship. Because of the often lethal erratic dose response clinical use of Phenester was not recommended.

NSC-129,943 2,6-Piperazinedione, 4,4'-propylenedi-, (±)-

ICRF 159 — Yale University School of Medicine

The oral administration of ICRF 159 to Beagles at various doses resulted in the production of moderate toxicologic changes which resembled the cytotoxic effects of numerous anticancer drugs with the more rapidly dividing cell populations affected. No totally unexpected signs developed and a dose of 20 mg/kg/day x 5 days appeared well tolerated and virtually nontoxic.

Overt signs included the appearance of anorexia, emesis and diarrhea. These observations were substantiated by histopathological findings of small intestinal edema, hemorrhage, cellular destruction and degeneration. In addition, the crypts of Lieberkühn were dilated, Peyers Patches appeared hyperplastic and inflammatory cells infiltrated the layers of the small intestine. The large intestine exhibited evidence of mild edema, cellular destruction, and hypersecretion of mucus.

Evaluation of bone marrow revealed that mature cells of both the erythyroid and myeloid series were decreased in numbers. Altered hematologic parameters included decreased white blood cell counts, depressed platelet production and occasional reticulocytopenia. Analysis of liver function tests indicated moderate elevations in SGOT and occasional increases in SGPT. No histopathologic changes were observed in the liver. Histopathologic evaluation of male reproductive organs revealed testicular degeneration and hypospermia with spermatid giant cell formation. The female reproductive system did not appear to be affected by this drug. The toxicologic observations of this study correlated well with previous reports of ICRF 159 toxicity from British investigators.

NSC-134,126 L-Glutaminase South Shore Analytical & Research Lab.,Inc.

L-Glutaminase, purified by the Nippon Kayaku Company from a Pseudomonas species and exhibiting roughly equal hydrolytic activity on both L-asparagine and L-glutamine, was examined toxicologically in Rhesus monkeys. The enzyme preparation was not pyrogenic but did cause a modest leukocytosis on the day of death; possibly it was contaminated with endotoxin. Hyperglycemia was noted in animals receiving 1,000 IU/kg/day x 3 days and 400 IU/kg/day x 5 days. The immunoreactive insulin of the animal receiving 400 IU/kg was low by day 3 and failed to rise with the hyperglycemia which followed an oral glucose load. Three doses of 1,000 IU/kg of this enzyme were lethal to one of two monkeys whereas a comparable dose of L-asparaginase from Escherichia coli has never been fatal to the monkey. Bowel hemorrhage was present, and may have resulted in this animal's death; however no significant lesions were detected histopathologically. Only the highest dose, 1,000 IU/kg administered daily for three days provoked a fatty liver. This was in sharp contrast to observations in the monkey receiving doses as low as 50 IU/kg of L-asparaginase from E. coli.

NSC-406, 021 9H-Purine-6-thiol, 9-β-D-arabinofuranosyl- (Ara 6MP) I. I. T. Research Institute

Large doses of this agent were necessary to produce lethality in mice.

Oral administration was considerably less toxic than intraperitoneal dosage. Mice dying after the drug treatment showed hydrothorax, erythemia of the small and large intestine and pulmonary congestion. In the Beagle, drug-induced deaths occured after single intravenous injections of 725 and 1,450 mg/kg and after five daily doses of 182 mg/kg/day. The major organ toxicity involved the liver, and was indicated by increased BSP retention, and elevated SGOT, SGPT and alkalaine phosphatase levels. The onset of these toxic signs was delayed in all cases and was confirmed by histopathologic evaluation. The response to the drug varied considerably from dog to dog. Occasionally, an animal given a lower dose showed toxic manifestations not observed at the immediately higher dose. Liver biopsies may have complicated the interpretation of some of these test results. Focal interstitial pneumonia, reported in several of the dogs, was thought to be compatible with a previously occurring spontaneous disease common to dogs and was in all probability not drug related. Evaluation of hematologic parameters revealed a dose-related leukopenia and occasional and rapidly reversible thrombocytopenia and anemia.

Polyvinylpyrrolidone (PVP) Hazlton Laboratories, Inc.

Polyvinylpyrrolidone (PVP) was evaluated for acute toxic effects by rapid intravenous administration to Beagles at doses of one, three and ten gms/kg. A cannula was implanted aseptically in the cephalic vein of each dog to facilitate the administration of the highly viscous solutions employed. Doses were calculated on the basis of concentrations of two, six and twenty percent PVP in saline and a constant volume of 50 ml/kg of body weight was administered.

Jumping or struggling during PVP administration was noted at all doses. These signs were followed by tremors and/or subconvulsive movements, defecation, salivation, depression and ptosis or puffy eyelids. One of two dogs at the high dose (10 gms/kg) exhibited a more severe reaction than other animals, including prostration, depressed corneal reflex, rigid muscles, and opisthotonus.

There were sporadic changes in transaminase and hematologic values which generally returned to normal by 48 hours post-dose, and remained essentially within normal ranges during the remainder of the 28 day observation period.

There were neither gross nor histopathologic changes in any tissues examined from dogs sacrificed at any dose.

Similar observations were made in the Rhesus after the intravenous administration of a single dose of 5 gms/kg.

These data appear to confirm that large doses of PVP can be administered to dogs and monkeys without serious toxicity. Many of the observed changes were probably due to the administration of large amounts (50 ml/kilo) of a highly viscous, hypertonic solution. There would appear to be very little hazard associated with the administration of small amounts of PVP as has been suggested for use in clinical dosage formulation.

PROTOCOL STUDIES IN PROGRESS:

On Going Studies

NSC-15200	Gallium (III) nitrate	South Shore Analytical & Research Lab.,Inc.
NSC-58514	Chromomycin A3	Mason Research Institute, Inc.
NSC-68075	Thalicarpine	Arthur D. Little, Inc.
NSC-71795	Ellipticine	South Shore Analytical & Research Lab.,Inc.
NSC-95441	Urea, 1-(2-chloroethyl(-3-(4-methylcyclohexyl)-1-nitroso- Methyl CCNU	Southern Research Institute
NSC-102,627	1-Propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride	South Shore Analytical & Research Lab.,Inc.
NSC-102,816	s-Triazin-2(1H)-one, 4-amino-1-β-D-ribofuranosyl- 5-Azacytidine	Arthur D. Little, Inc.
NSC-106,977	L-Asparaginase, from Erwinia Carotovora	South Shore Analytical & Research Lab.,Inc.
NSC-109,724	1,3,2-Oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino] tetrahydro-, 2-oxide	Arthur D. Little, Inc.
NSC-119,875	Platinum, diamminedichloro-, cis-	Mason Research Institute, Inc.
NSC-129,943	2,6-Piperazinedione, 4,4'-propylenedi-, (+)- ICRF 159	Yale University School of Medicine

SPECIAL STUDIES:

NSC-740	Methotrexate	University of Texas at Austin
NSC-3053	Actinomycin D	
NSC-8806	Melphalan	
NSC-19893	5-Fluorouracil	
NSC-49842	Vinblastine	

To study the toxicity following chronic administration of drug (5-year study).

NSC-742	Azaserine	Mason Research Institute, Inc.

To determine whether ethyl diazoacelate is a toxic metabolite of Azaserine. Comparative LD_{50}'s.

NSC-1895	Guanazole	Arthur D. Little, Inc.

To study the influence of infusions in the Beagle over varying periods of time (48 and 72 hr) on the overall toxicity.

NSC-3053	Actinomycin D	I. I. T. Research Institute

To establish highest non-toxic and lethal doses in Beagles and Rhesus monkeys using the fractions and multiples of the established clinical dose and schedule.

NSC-58514	Chromomycin A3	Mason Research Institute, Inc.

To study the comparative toxicity of the Japanese vs. NCI clinical formulation.

NSC-63878	Cytosine, 1-β-D-arabinofuranosyl-hydrochloride	Arthur D. Little, Inc.

To study the influence of dosage schedule in the dog on the toxicity of Ara-C.

NSC-77213	Procarbazine hydrochloride	I. I. T. Research Institute

To establish highest non-toxic and lethal doses in Beagles and Rhesus monkeys using fractions and multiples of the established clinical schedule and dose.

NSC-83291	L-Asparagine	Mason Research Institute, Inc.

To study in the normal Beagle the reproducibility of clearance of 4-C-^{14}-L-asparagine.

NSC-85998 Streptozotocin South Shore Analytical & Research Lab.,Inc.

To determine if Streptozotocin + Nicotinamide causes pancreatic islet cell tumors in the Beagle. To determine whether renal tubular acidosis in the dog is induced by Streptozotocin as it is in man. (CCR. 54 (6): 457, 1971)

NSC-85998 Streptozotocin South Shore Analytical & Research Lab.,Inc.
NSC-13128 Nicotinamide

To follow the pathogenesis of pancreatic islet cell tumors in mice following a single treatment with Streptozotocin and Nicotinamide.

NSC-102,627 1-Propanol, 2,3'-iminodi-, dimethanesulfonate (ester), hydrochloride South Shore Analytical & Research Lab.,Inc.

To explore the possibility of testicular toxicity and tumors occurring after drug treatment.

NSC-109,229 L-Asparaginase Mason Research Institute, Inc.

To determine the rate of biosynthesis of L-asparaginase in the whole animal by means of infusions of ultra-low doses of L-asparaginase (2 dogs).

NSC-109,229 L-Asparaginase Arthur D. Little, Inc.

To follow the cellular fate of L-asparaginase in the normal and immune mouse.

NSC-109,229 L-Asparaginase (DONV inactivated) South Shore Analytical & Research Lab.,Inc.

To ascertain whether covalently inactivated (DONV-titrated) L-asparaginase can cause hepatotoxicity.

NSC-109,229 L-Asparaginase Mason Research Institute, Inc.

To develop in the cat and/or mouse a model for studying the neurotoxicity of L-asparaginase.

NSC-109,229 L-Asparaginase South Shore Analytical & Research Lab.,Inc.
NSC-3053 Actinomycin D

To determine if toxicity of the enzyme is augmented when administered in combination with Actinomycin D.

NSC-109,229 L-Asparaginase from Escherichia coli — Mason Research Institute, Inc.

NSC-106,977 L-Asparaginase, from Erwinia carotovora

To study in mice the effect of E. coli and Erwinia carotovora L-asparaginase on brain levels of L-asparagine and L-glutamine.

NSC-109,724 1,3,2-Oxazaphosphorine, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-, 2-oxide — Arthur D. Little, Inc.

To investigate the effect of sulfhydryl-containing compounds on isophosphamide-induced cystitis in the dog.

NSC-123,127 Adriamycin, hydrochloride — University of Texas at Austin

To study the pathogenesis of the cardiac toxicity of this Daunomycin analog.

NSC-125,066 Bleomycin — Mason Research Institute, Inc.

To study the influence of route of administration (IM vs. IV) on drug toxicity.

NSC-125,066 Bleomycin — Mason Research Institute, Inc.

To study the pathogenesis of Bleomycin integumental toxicity by means of sequential skin biopsies taken for histopathologic and enzymatic studies. In collaboration with Drs. Kyser and Friedland.

NSC-125,066 Bleomycin — Mason Research Institute, Inc.

To study the influence of Bleomycin on the pulmonary compliance of the Beagle.

NSC-125,066 (under subcontract to Rhode Island) to measure 9 selected tests of pulmonary function in Beagles given Bleomycin subacutely.

NSC-125,066 Bleomycin — Mason Research Institute, Inc.

To define a parameter (cutaneous toxicity) which may serve as a warning of impending pulmonary toxicity.

NSC-125,066 Bleomycin — Mason Research Institute

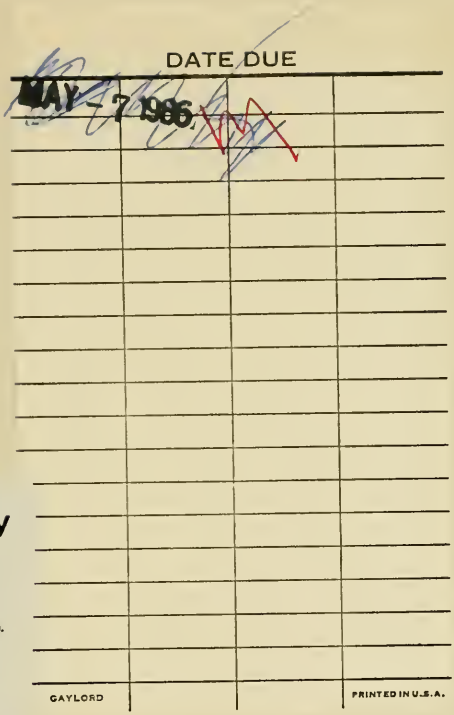
To develop small animal models (mouse and hamster) for study of Bleomycin-induced pulmonary toxicity, in order that antidotes and prophylaxes can be studied with statistically significant numbers of animals.

NSC-125,066 Bleomycin Mason Research Institute, Inc.

To explore the possibility of preventing Bleomycin pulmonary toxicity by the concomitant administration of Prenisolone (or comparable corticosteroid derivative).

NSC-136,519 β-Alaninamide, sulfate (2:1) Mason Research Institute, Inc.

To determine the intravenous LD_{50} in mice of the L-asparagine analogs.



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